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COMPARATIVE STUDIES OF WINTER HARDINESS IN WHEAT

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COMPARATIVE STUDIES OF WINTER HARDINESS IN WHEAT [1]

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THE WINTER-HARDINESS PROBLEM IN WHEAT

Winterkilling is practically the only factor which limits the growing of winter wheat in northern regions, especially in the north-central part of the United States. The losses due to winterkilling in the United States, the methods of reducing winter injury, and the hardiness of certain varieties have been shown previously (*10*, *11*, *26*, *28*).[3]

Several difficulties are encountered in determining the hardiness of wheats by field methods. Winterkilling of wheat plants in the field is very irregular, and complete killing or complete survival of the plants frequently occurs. Repeated field tests are necessary to measure hardiness in varieties. Poorly adapted varieties often show a low winter survival even though they are known to be resistant to cold (*14*). Frequently it is not low temperatures, but drought, soil blowing, soil heaving, and smothering by ice which are responsible for the killing of winter-wheat plants. The low correlation between the survival of winter-wheat hybrid strains and that of selections from them was shown by Vassar.[4] In the writer's experiments no significant correlations were obtained between the survival of hybrid strains and selections from them. A laboratory method for measuring winter hardiness, which would be more accurate than field observation, therefore, is greatly needed.

The complexity of the problem and the differences between varieties have made positive conclusions regarding the nature of hardiness rather difficult. Many characters possessed by hardy wheat varieties also are found in nonhardy varieties or they do not occur in rye, which is hardier than wheat. The hard red winter wheats are the hardiest group known. These wheats are characterized by narrow, dark-green, prostrate leaves in the seedling and rosette stages. The narrow leaves seem to be fairly well associated with hardiness in certain American winter wheats but not necessarily in all groups of wheat. The dark color and prostrate position of juvenile leaves also are not definitely related to hardiness. Any relationship between hardiness,

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[2] The writer wishes to express his thanks to Dr. R. A. Gortner, professor of agricultural biochemistry at the University of Minnesota, for his help in outlining these experiments and in providing facilities for carrying them out; to Dr. H. K. Hayes, professor of plant breeding, and other members of the staff of the Minnesota Agricultural Experiment Station, for materials and equipment furnished and suggestions given during the course of these investigations; and to Dr. Robert Newton, professor of field husbandry at the University of Alberta, for supplying the writer with some of his experimental results on winter hardiness of wheat in advance of their publication by him.

[3] Reference is made by number (italic) to "Literature cited," p. 532.

[4] VASSAR, L. P. FIELD AND LABORATORY METHODS FOR A DETERMINATION OF WINTER HARDINESS IN WINTER-WHEAT HYBRIDS. Unpublished thesis, submitted to the Graduate School of the University of Minnesota in partial fulfillment for the degree of master of science, June, 1924.

all anatomical characters, and nearly all physiological characters probably could be disproved by the proper selection of a limited number of cereal varieties.

The recent work of Newton (*34*, *35*, *36*) shows, however, some important physiological characteristics of several of the hardy wheat varieties, which might be used in measuring the hardiness of varieties and hybrids.

REVIEW OF LITERATURE ON WINTER HARDINESS IN PLANTS

CAUSES OF WINTERKILLING

In spite of abundant data to the contrary the belief of the old Greek philosophers and of Duhamel and Buffon as late as 1740 (*13*) that frost injury in plants is due to rupture caused by growing ice crystals within the cells has not yet been entirely dissipated. Göppert (*15*), Sachs (*44*), Müller-Thurgau (*32*, *33*), Cavallero (reported by Abbe (*1*)), Molisch (*30*), and Wiegand (*56*) observed that ice formation takes place mostly in intercellular spaces and that the rupture of cells by freezing rarely occurs. Müller-Thurgau (*32*, *33*) reported thta ice formation takes place within the cell only on rapid cooling, and Cavallero (*1*) stated that, even though ice is formed within the cells, the protoplasm never freezes. Molisch (*31*), Müller-Thurgau (*32*, *33*), and Wiegand (*56*) stated that frost injury was due to the withdrawal of water from the cells by freezing.

Other workers, including Cavallero (*1*) and Schindler (*51*), believed that frost resistance was due to chemical peculiarities of the cell protoplasm. Frost injury was believed by Gorke (*16*), Lidforss (*24*), Schaffnit (*48*), and Schander and Schaffnit (*50*) to be due to the precipitation of cell proteins, through salting out by the increased concentration of the cell sap after water had been withdrawn in freezing. Voigtländer (*55*) and Chandler (*9*) disagreed with this theory, the latter finding that plants grown with an abundance of nutrient salts are hardier and their protoplasm less easily precipitated than is the case with plants grown with a poorer supply of nutrients. The higher sap concentration of the better nourished plants, however, probably tends to increase imbibition; in which case Chandler's objection is not necessarily valid.

Mez (*29*) believed death to be due to cooling a plant below its specific minimum and held that the prevention of undercooling during freezing sometimes keeps the temperature of a plant above its specific minimum. Apelt (*4*) found that undercooling will not continue long, while Voigtländer (*55*) stated that undercooling rarely occurs in nature and is not related to the killing point or the death of the plant. Sachs (*44*) believed death from cold resulted from several factors, including shrinkage and disorganization of the cell following ice formation, ice expansion injury, and destruction by thawing after freezing. Detmer (*12*) stated that plant tissues were killed during freezing, because a change in the color of the chlorophyll could be observed.

The recent view appears to be that frost killing consists in the withdrawal of water from the cells to form ice in the intercellular spaces, followed by the precipitation of the protoplasm, due chiefly to the increased concentration of the cell sap.

THE NATURE OF COLD RESISTANCE

The factors which frequently have been reported concerned with cold resistance include low moisture content, increased sugar content[5] (*2, 3, 4, 18, 24, 36, 48*), increased sap concentration, increased colloidal material, and a long vegetative period (*8, 51*), as well as certain morphological characters of wheat, such as narrow or prostrate leaves (*8, 45*) and large cells (*5*).

The relation of low moisture content to hardiness in wheat was shown by Sinz (*53*) and Newton (*35*). The same relationship was shown in apple twigs by Shutt (*52*) and by Beach and Allen (*7*), in peach twigs by Johnston (*21*), in plum twigs by Strausbaugh (*54*), and it has been indicated for vegetable plants by Rosa (*43*).

Although Maximow (*27*) and Chandler (*9*) found that increasing the sap density of plants increased the resistance to freezing more than could be accounted for by the increased osmotic pressure, Salmon and Fleming (*46*), Pantanelli (*40*), and Baroulina (*5*) found no definite relation between osmotic concentration of the sap and the cold resistance of cereals. The failure to find a relation probably was due to the selection of varieties, the attempt to compare different crops, or the time of collecting samples. The method of extraction influences the freezing point of the sap, and it may differ considerably from the freezing point of the tissues, as shown by Newton and others (*38*) and by Wright (*58*).

Imbibitional pressure due to hydrophilic colloids, as measured by the quantity of juice expressed from wheat leaves, was shown by Newton (*34, 35*) to be closely related to hardiness, the more hardy varieties yielding the lesser quantity of juice. The relation of total solids and bound water to hardiness was pointed out by Newton and Gortner (*37*).

Rosa (*43*) reported pentosan content to be related to hardiness in vegetable plants, but Newton[6] did not find this relationship in wheat. Recent experiments by McGinty (*25*) indicate that the methods used by Rosa would not actually measure pentosan content.

Zacharowa (*59*) reported the most cold-resistant tissues to be the most alkaline and to contain a lower salt concentration and a higher protein content. Kolkunov (*23*) reported that xerophytic wheat varieties resist injury from low temperatures.

Newton (*35*) suggested that, because of the better retention of sugars, hardy cereals may have a lower rate of respiration than nonhardy ones, which has been shown by Govorov (*18*) to be the case.

LETHAL TEMPERATURES FOR CEREAL PLANTS

Schaffnit (*49*) found that wheat seedlings were injured very little at $-5°$ to $-10°$ C. but were considerably injured at $-15°$ to $-20°$ C. Both the degree and the duration of cold affect the extent of killing. Klages (*22*) exposed wheat plants to cold for short periods and obtained results similar to those of Schaffnit. Rye seedlings three or four days old were found by Zacharowa (*59*) to be killed at $-7.8°$ but not at $-2.9°$ C. At $-5.75°$ and $-3.9°$ C. the seedlings were killed except at the root tips. Buhlert (*8*) froze the juices of spring

[5] NEWTON, R. THE NATURE OF WINTER HARDINESS IN CROP PLANTS. Progress Reports to the Honorary Advisory Council for Scientific and Industrial Research (Canada). (Typewritten reports, May, 1924, and May, 1925, furnished the writer by courtesy of Dr. Robert Newton.)

[6] NEWTON, R. Op. cit.

barley, winter barley, and winter rye and found at −7.0°, −12°, and −15° C., respectively, a precipitate consisting of protein with traces of lime and phosphoric acid.

PROBLEMS INVESTIGATED

The chief object of the investigations undertaken by the writer was to determine the most accurate and feasible methods for measuring winter hardiness in wheat varieties and hybrids. Four general problems were studied: (1) Laboratory investigations of factors for hardiness; (2) determination of lethal temperatures for wheat; (3) hardiness of plant parts; and(4) hardiness in wheat hybrids. Other objects of the investigations were to determine further the processes of hardening, the causes of winterkilling, and the inheritance of winter hardiness in wheat plants.

LABORATORY INVESTIGATIONS OF FACTORS FOR HARDINESS

The factors which were investigated in the laboratory studies included the moisture content and imbibitional pressure of the leaves, total solids, freezing-point depression, and bound water in the expressed juice or sap, the seasonal changes in these factors, and the rate of respiration of wheat varieties at low temperatures.

MATERIALS AND METHODS USED

Four varieties of wheat—Minhardi, Kanred, White Winter, and Marquis—were used in these investigations. Minhardi has been shown (*11*) to be the hardiest winter-wheat variety grown in the United States. Kanred, an important hard red winter variety, is less hardy than Minhardi. White Winter was selected because it appeared to be among the least hardy of the winter wheats. Marquis, a spring wheat, was used for comparison with the winter wheats because of its relative hardiness among the spring wheats. Swedish (Minnesota No. 2) rye was included in the experiments because it was known to be considerably more hardy than Minhardi wheat and to determine whether the same physiological factors for hardiness occur in wheat and rye.

The moisture content of the leaves was determined from samples of approximately 10 grams, weighed before and after drying 24 hours or more in an oven at 100° C. The imbibitional pressure of the leaves was determined by measuring the quantity of juice or sap per gram of green tissue expressed by a hydraulic press at pressures of 50, 150, 250, and 350 kgm. per square centimeter. Samples, usually consisting of 25 to 75 gm. of tissue, were wrapped in pieces of boiled unbleached muslin 5 inches square. They were then placed in a steel press bowl and the hydraulic pressure applied to a piston in the bowl.

The percentage of total solids in the sap was determined by means of the Abbe refractometer, the sugar scale being used as a basis for estimating solids, as suggested by Gortner and Hoffman (*17*). Freezing-point depressions were determined with a Beckmann apparatus, a salt-and-ice bath being used. The percentage of bound water in the sap was ascertained by the method of Newton and Gortner (*37*), by determining the freezing-point depression of the

sap before and after adding pure sucrose in a quantity equivalent to a molecular concentration of sucrose in the water of the sap. A quantity of sap containing 10 gm. of water, as determined by the percentage of total solids, was weighed out, 3.422 gm. of sucrose added, and the freezing-point depression then determined.

For example, a sample of sap showed a freezing-point depression of 1.51° C. With a quantity of added sucrose necessary to give a molar concentration, it had a freezing-point depression of 3.86° C., which, minus 1.51°, equals 2.35°, the excess freezing-point depression. Of this excess, 2.085° was due to the sucrose, which in solution forms sucrose hexahydrate. Then 2.35° minus 2.085° equals 0.265°, which is the excess freezing point due to water being bound by colloids in the sap so that it can not dissolve the sucrose. Dividing 0.265 by 2.35 and multiplying the result by 89.2 gives 10.1 per cent of bound water. The figure 89.2 shows the quantity of free water in 100 c. c. of a molar solution of sucrose in which 6 molecules or 10.8 per cent of the water is taken up to form sucrose hexahydrate.

The lethal temperatures for wheat plants were determined by subjecting the plants growing in pots to freezing, usually for 24 hours at known temperatures, and then returning the pots to the greenhouse and noting the percentage of plants which recovered. The rate of respiration was measured by drawing a stream of air free from carbon dioxide through a closed chamber covering the wheat leaves, which chamber was sealed off from the laboratory air and from the soil in which the pots were growing by a half-and-half mixture of vaseline and liquid paraffin. The air was caused to flow through the chamber by equal suction and pressure at the rate of 18 liters per hour. Five volumes of air passed through the chambers during each half hour of aspiration, as the capacity of the chambers was 1.8 liters. The chambers were aspirated for half-hour periods whenever a sufficient quantity of measurable carbon dioxide had accumulated. The carbon dioxide given off by the plants was collected in bead-absorption towers containing a standard solution of barium hydroxide, and the excess of alkali was titrated with HCl, using phenolphthalein as an indicator. The acid solution was of such strength, N 0.04545, that each cubic centimeter was equivalent to 1 mgm. of CO_2. The temperatures during respiration were kept constant to within about 0.1° C. by placing the potted plants in the chambers of an ice-cream cabinet which were electrically heated by means of lamps, to keep the temperatures in the chambers above that of the surrounding bath. The temperatures were controlled by thermoregulators. (Fig. 1.)

The wheat used in these investigations of the tissue-fluid properties was sown in 8-foot rows on fall-plowed land. The sowing was done by hand, September 11 and 12, in 1923, at the rate of 5 gm. per row or 1 bushel per acre. In 1925 it was sown with a hand planter on August 27, at the rate of 5.5 gm. per row. The rye was sown September 21 in 1923, but in 1925 it was sown on the same date as the wheat. Duplicate plots were sown consisting of 25 rows of each variety in 1923 and 50 rows in 1925. In 1923, delayed rains caused a slow growth of wheat, and the plants had reached only the three-leaf stage when the first samples were gathered October 19. Growth continued, however, until the latter part of November. In 1925,

much better early growth occurred, but there was not much increase in dry matter after October 15. Heavy wrapping paper was placed each year over the wheat plants after they had stopped growing to keep the snow from them. This facilitated the gathering of samples when the ground was covered with snow.

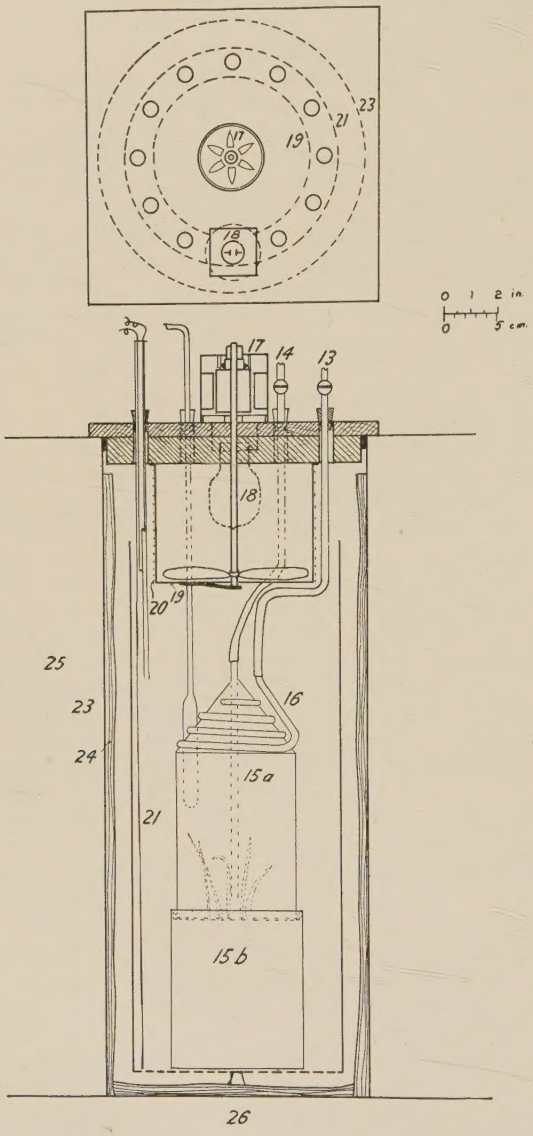

FIG. 1.—Diagram of the constant-temperature apparatus used for the respiration experiments: 13, Tube for ingoing air; 14, tube for outgoing air; 15a, sheet-metal respiration chamber; 15b, earthenware jar containing growing plants with the soil surface sealed off; 16, coil of copper tubing for cooling air before it enters respiration chamber; 17, fan motor; 18, socket and light for supplying heat; 19, support for fan shaft bearing and fan guard; 20, cylindrical wire screen surrounding the fan blade; 21, sheet-metal cylinder; 23, wall of cell of freezing cabinet; 24, asbestos lining for insulating the cell; 25, fluid of the freezing cabinet; 26, cork insulation of freezing cabinet

Duplicate samples of each variety were collected from adjoining rows at intervals of three or four days during the autumn and at greater intervals after December 15. Nearly all of the samples were collected between 10 a. m. and noon. The plants were cut off at the surface of the soil with shears and placed in screw-top bottles. They were then taken to the laboratory, and about 10 gm. of tissue was removed from the top of each bottle for moisture determinations. The remainder of each sample was weighed and taken immediately to the "hardening" room, located in the dairy building, where it was maintained in a frozen condition. Samples which were frozen when collected were not permitted to thaw. The hardening room varied in temperature usually between −12° C. (10° F.) and −23° C. (−9° F.) but dropped to the latter figure nightly. After they had been left 24 hours or more in the hardening room, the samples were returned to the laboratory and pressed. In 1923, one of the duplicate samples of each variety was pressed soon after it was collected and

without preliminary freezing. In 1925, both samples of each variety were frozen.

The daily maximum and minimum temperatures recorded at St. Paul, Minn., during the fall and winter months from October to February, inclusive, in 1923–24 and 1925–26, are shown in Table 1. These data are given because of their relation to the hardening-off process in the wheat plants under field conditions, this relation being reflected in the data obtained on the different sampling dates as hereafter given. In 1923 the first freeze occurred on October 19, the date of the first collection of winter wheat from the field, but the wheat plants apparently had not been frozen. In 1925 the first collection was made on October 12, after light frosts had occurred, but while the plants were still in an actively growing condition.

TABLE 1.—*Daily maximum and minimum temperatures at University Farm, St. Paul, Minn., during stated fall and winter months of 1923–24 and 1925–26*

Day	Temperature (° F.)																			
	1923–24										1925–26									
	Oct.		Nov.		Dec.		Jan.		Feb.		Oct.		Nov.		Dec.		Jan.		Feb.	
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
1	66	47	52	23	43	19	−1	−19	32	28	57	43	50	27	35	12	36	20	21	9
2	72	42	45	40	35	11	10	−3	41	29	63	48	50	28	39	32	38	21	25	10
3	71	44	52	35	43	23	−2	−19	35	26	59	44	48	33	36	32	30	20	27	13
4	61	42	48	28	44	27	−9	−22	26	9	52	42	53	29	33	12	37	30	20	11
5	59	36	52	30	44	16	−6	−27	11	3	56	37	40	24	13	7	35	24	37	10
6	62	38	58	32	40	26	36	−6	12	3	51	31	31	19	20	11	26	11	37	24
7	62	44	43	26	45	26	31	16	20	3	41	38	25	12	32	14	18	4	41	16
8	59	41	41	22	45	29	32	22	26	1	42	37	29	8	39	25	22	6	35	29
9	75	48	58	31	33	21	30	20	28	18	39	30	49	23	28	21	22	5	32	19
10	77	52	59	37	35	20	23	15	37	12	45	26	52	30	33	20	33	22	24	8
11	71	54	60	31	48	21	15	6	33	24	59	41	56	30	33	30	30	−5	31	11
12	58	43	49	41	42	17	6	−8	32	17	56	36	52	27	34	13	23	−7	33	27
13	53	39	50	45	25	10	5	−11	39	27	58	43	47	30	21	11	22	2	33	26
14	52	34	48	43	41	14	13	−14	37	18	52	42	32	30	21	8	36	16	29	12
15	65	35	45	38	47	30	24	8	16	3	58	37	32	27	25	14	28	8	17	6
16	66	53	38	30	46	33	8	−16	26	12	44	32	30	22	19	10	36	25	41	5
17	69	53	55	28	52	32	6	−16	25	17	44	27	49	22	12	5	37	21	32	15
18	53	37	47	33	54	28	10	−1	22	14	33	29	50	34	22	2	38	22	27	9
19	44	30	50	28	49	34	2	−9	24	16	38	30	45	32	25	17	26	4	25	11
20	47	30	51	37	43	38	−6	−20	23	10	35	30	61	34	24	15	12	−5	21	12
21	52	27	38	20	44	35	14	−21	16	2	47	26	45	26	15	9	4	−13	21	11
22	58	31	37	16	36	28	34	14	21	−2	32	27	26	16	16	11	5	−17	23	10
23	60	34	36	28	33	23	17	1	23	5	37	31	36	16	20	9	24	−5	43	19
24	55	36	41	28	33	23	29	0	27	6	41	32	43	27	17	8	2	−9	36	30
25	42	37	50	33	35	25	0	−15	36	11	42	30	37	23	10	−3	17	−6	37	31
26	44	39	33	26	42	26	0	−11	43	16	37	25	38	16	−2	−13	23	−1	31	16
27	42	34	31	19	35	13	22	−8	44	19	26	20	18	5	5	−10	13	−13	34	12
28	43	32	41	19	26	9	36	17	39	26	24	17	21	5	6	−6	8	−20	48	31
29	34	22	46	27	20	8	40	24	33	28	23	13	23	19	14	−8	34	8	----	----
30	32	19	46	23	8	0	37	23	----	----	27	8	31	----	16	1	36	25	----	----
31	44	24	----	----	11	0	35	28	----	----	42	24	----	----	32	7	31	23	----	----
Mean	56.4	38.0	46.7	29.9	38.0	21.5	15.8	−1.7	28.5	13.8	43.9	31.5	40.0	23.2	22.4	10.2	25.2	7.0	30.8	15.8

The temperatures in 1923 continued rather mild, and the wheat continued to grow until well into November and resumed growth again during the few days following December 15. During the night of December 26 the temperature dropped abruptly and continued low until January 6, 1924. During this period the growth

of wheat stopped and the plants remained frozen and were covered with snow. The sudden change of temperature caught the plants in an unhardened condition, but in spite of this they survived the minimum temperature of −33° C. (−27° F.) of January 5 with little or no injury. This was due to the 3-inch covering of snow. Thermograph records of the air temperature and the soil temperature just below the surface on a plot a few rods from the wheat plants were kept by A. C. Arny, and some of the results were shown to the writer. During the first two weeks of January, when the air temperature dropped to a minimum of −33° C. (−27° F.) and rose to a maximum of about +2° C. (+36° F.), the soil temperature remained practically constant at −2° C. (+28° F.), which is not fatal to any variety of winter wheat. After January 1 the wheat remained covered with some snow until spring.

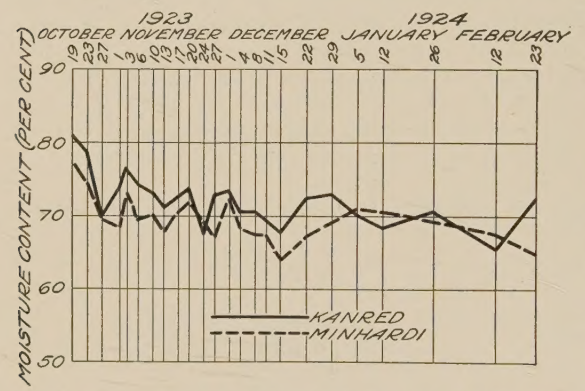

FIG. 2.—Moisture content of Kanred and Minhardi wheat leaves collected at intervals during the autumn and winter of 1923–24, University Farm, St. Paul, Minn.

In the autumn of 1925 the wheat apparently continued growth until the latter part of October and remained vegetatively active until the last week in November. The temperatures during December and January were relatively low, and there was some snow on the ground continuously. The wheat plants remained frozen nearly all of the time from the latter part of November until early in March, although the plants collected on January 16 had thawed somewhat.

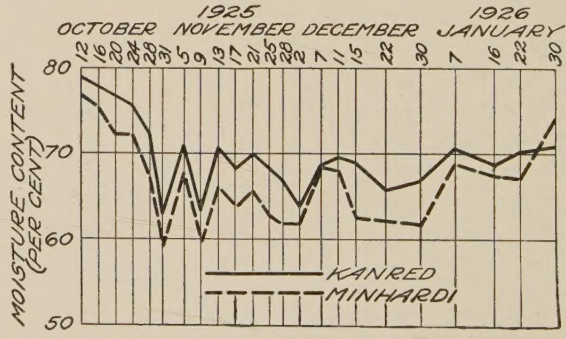

FIG. 3.—Moisture content of Kanred and Minhardi wheat leaves collected at intervals during the autumn and winter of 1925–26, University Farm, St. Paul, Minn.

The official temperatures recorded were taken with instruments placed in a shaded inclosure, while in the field on clear days the sunshine frequently caused the plants to thaw and take up water from the soil, even when the maximum temperature recorded in the instrument shelter was below freezing. The wheat plants thus were able to recover water to replace that lost by freezing and evaporation, apparently changing their imbibitional power during periods in which, on the basis of recorded temperatures, they would be expected to remain frozen and unchanged.

The winter of 1923–24 might be considered an abnormal one, on account of the long-continued growing weather and the high temperatures in December, followed by very low temperatures in early January. The absence of low temperatures except when the ground was covered with snow permitted almost complete survival of winter wheat. The season of 1925–26, on the other hand, was more nearly normal, as temperatures tended to decrease gradually until the soil was frozen and covered with snow at the end of November. The conditions prevailing during that season and their effects on winter wheat were about such as might be expected in most seasons in sections having a climate similar to that of St. Paul, Minn. Rather heavy winterkilling occurred in the winter-wheat nurseries.

RESULTS OBTAINED

The data obtained from the laboratory investigations of factors for winter hardiness in wheat are shown in detail in the following pages. In the discussion following the presentation of these and other experimental results, the relations of the various factors concerned with hardiness and hardening are considered.

Moisture Content

Data showing the average moisture content of duplicate samples of wheat leaves collected during the two seasons are given in Table 2 and are shown graphically in Figures 2 and 3. The moisture content of the leaves of the different varieties was about 80 per cent during early October, when the plants were actively growing. With the approach of cold weather the moisture content dropped to about 70 per cent. Most of this decrease resulted from the wilting of the tissues following freezing. After each freeze some of the lost moisture was recovered. This explains the wide fluctuation in moisture content shown in Table 2. Dead tissues were not entirely eliminated from the moisture samples. The separation of the dead tissue is very difficult when considerable quantities of partly wilted tissue, are present. It is not known just how little moisture the tissues could contain without being wilted in appearance, but, in general, all samples collected in late autumn or winter which showed less than 68 to 70 per cent moisture were at least partly wilted. The hardy varieties could contain less moisture than the less hardy varieties without being wilted.

From the data of Table 2 it is evident that the Kanred variety had a higher moisture content than the Minhardi on all but five dates. These exceptions followed severe freezes in which Kanred was more injured than Minhardi. The difference in moisture content may well be considered a measure of hardiness in which the hardier variety has the lower moisture content. White Winter wheat, when not too badly wilted, showed a higher moisture content than either Kanred or Minhardi. Rye tends to have a high moisture content, even though it is extremely hardy. This, however, does not disprove the relationship in wheat, which belongs to another genus.

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TABLE 2.—*Moisture content of the plants of four varieties of wheat and one variety of rye collected on stated days during the seasons of 1923–24 and 1925–26, University Farm, St. Paul, Minn.*

Date collected	Moisture content (per cent)				
	Minhardi	Kanred	White Winter	Marquis	Minnesota No. 2 rye
Season of 1923–24					
October:					
19	77.8	81.1			
23	74.7	78.9			
27	69.6	69.8			
November:					
1	68.6	73.7			
3	73.2	76.9	76.0		76.3
6	69.7	74.6			
10	70.2	73.4	72.6		75.2
13	67.8	71.3			
17	70.6	72.9	73.6		74.2
20	71.9	73.9			
24	69.1	67.8	73.4		69.6
27	66.9	72.8			
December:					
1	72.5	73.6	73.2		72.0
4	68.7	70.9			
8	67.5	70.9	69.1		
11	67.4	69.7			
15	64.2	67.9	57.3		
22	67.2	72.5	67.5		
29	69.2	73.1	72.7		
January:					
5	71.1	70.3	66.7		
12	70.7	68.5	68.6		
26	69.4	70.8	75.2		
February:					
12	67.7	65.7	69.2		
23	65.0	72.4	64.5		
March:					
8	56.7	63.7	64.3		
27	60.9	57.0			
May:					
17	75.1	78.7			
Average [a]	68.677 ±0.512	71.219 ±0.590	69.593 ±0.847		
Season of 1925–26					
October:					
12	76.8	78.7	81.2	81.8	80.2
16	75.4	77.5	80.5	80.9	78.4
20	72.2	76.7	78.4	78.5	77.4
24	72.1	75.5	77.2	77.0	74.8
28	67.4	72.1	72.9	69.5	72.1
31	59.3	62.9	64.0	65.4	64.9
November:					
5	67.8	70.8	71.0	71.8	72.7
9	59.6	63.2	64.5	57.6	65.5
13	66.0	70.8	70.5	66.1	71.1
17	63.7	68.1	68.1	60.4	68.4
21	65.6	69.9	69.8	68.4	70.0
25	62.7	68.1	65.5	60.7	71.1
28	61.7	66.9	66.2	62.4	70.7
December:					
2	61.9	63.8	62.0	59.2	67.7
7	68.5	68.7	66.0	64.7	71.9
11	68.0	69.7	68.6	69.1	72.6
15	62.6	69.0	65.1	61.2	73.1
22	62.3	65.8	62.3	62.2	71.3
30	61.8	66.6	57.5	58.7	70.4
January:					
7	68.8	70.6			72.5
16	67.4	68.8	65.1	66.3	69.1
22	67.0	70.2			68.6
30	74.0	70.9	76.2	60.2	67.9
May:					
4	73.5	77.7			79.2
Average [b]	66.635 ±0.682	69.796 ±0.576	69.171 ±0.933	66.767 ±1.071	71.409 ±0.518

[a] Not including samples collected Oct. 19.

[b] Not including samples collected May 4.

The data on moisture content for 1925–26 are more complete than those for 1923–24. In 1925, on October 12, 16, 20, and 24, the moisture contents of Marquis and White Winter were distinctly higher than those of the hardier wheats, Minhardi and Kanred. Rye again showed a moisture content similar to that of the nonhardy varieties. During the autumn of 1925 the moisture content of all varieties decreased until about December 2, when a certain recovery occurred. The moisture contents of White Winter and Marquis leaves were relatively low after October 28. During this season the moisture contents again showed large fluctuations due to freezing and thawing, with consequent wilting and recovery. The higher moisture content of Kanred, as compared with Minhardi, was maintained throughout the season except on January 30. It is evident from this and from the behavior of White Winter and Marquis wheats that varieties somewhat similar in hardiness tend to fluctuate together, while widely different varieties are dissimilar because they are affected in widely different degrees by freezes or by their ability to recover. True comparisons in moisture content can be obtained only when the plants are not affected by freezing and wilting. On October 12, 16, and 20, the four wheat varieties had moisture contents in the inverse order of their hardiness. After that time this relationship did not exist. The lowest moisture contents of the nonhardy varieties were less than the lowest moisture contents determined for the hardy varieties, because the former contained more wilted and dried leaves.

Duplicate samples of 10 varieties of winter wheat and one variety of rye were collected for moisture determinations, from nursery rows, on May 31, 1924. The plants at that time had almost reached the jointing stage. The moisture-content data are given in Table 3 with the wheat varieties arranged in the order of their hardiness. Rye, although much hardier, had a moisture content higher than any of the wheats. The four hardiest wheat varieties—Minhardi, Buffum No. 17, Minturki, and Odessa—had moisture contents lower than the less hardy varieties, although the order of the moisture contents and the relative hardiness of these varieties among themselves was inverse to that of all varieties as a group. The remaining varieties with somewhat higher moisture contents also failed to show a definite relation between hardiness and moisture content. Differences in earliness and consequent stages of growth, however, may have affected the results.

On November 16, 1925, triplicate samples of five varieties of wheat were collected from the border rows of the varietal plots at University Farm. Collections were not made early in October, because sufficient growth had not been attained, and the samples collected November 9 were too badly wilted to show reliable results. The moisture contents of the five varieties, listed in their order of hardiness, are shown in Table 3. While again no definite relation between hardiness and moisture content were shown, Red Rock, the least hardy variety, contained decidedly more moisture than any other variety, and Kanred, the second least hardy variety, showed the second highest moisture content.

TABLE 3.—*Average moisture content of samples of the plants of varieties of winter wheat and rye collected at University Farm, St. Paul, Minn., and listed in the order of average hardiness*

Duplicate samples collected May 31, 1924				Triplicate samples collected Nov. 16, 1925	
Variety	Moisture content	Variety	Moisture content	Variety	Moisture content
	Per cent		*Per cent*		*Per cent*
Minhardi	74.9	Kanred	76.1	Minhardi	79.0
Buffum	73.4	Kharkof	76.7	Minturki	77.9
Minturki	73.3	Blackhull	76.4	Turkey	77.2
Odessa	72.7	Nebraska No. 28	75.5	Kanred	79.2
Padui	76.9	Minnesota No. 2 rye	81.2	Red Rock	82.1
Turkey	75.7				

An experiment to determine the rate at which wheat plants dry out at low temperatures was conducted by the use of artificial refrigeration. At the beginning of the experiment, samples taken from pots of Kanred and Minhardi wheat growing in the greenhouse contained 75.5 per cent and 73.4 per cent of moisture, respectively. Other pots containing plants of these varieties were then exposed successively to the following temperatures during the periods named: 1° C. for 24 hours, −8° C. for 36 hours, and −12° C. for 8 hours. At the end of this period Kanred contained 73.3 per cent and Minhardi 72.8 per cent of moisture. The remaining plants were then maintained at temperatures between −2° and −4° C. for one week. Under these conditions the plants were thawed, but the soil remained frozen. At the end of the week the moisture content of the Kanred samples was reduced to 36.6 per cent and that of the Minhardi samples to 41.6 per cent. These latter samples of the two varieties consisted of the remaining plants from the same pots from which the previous samples had been taken. Duplicate pots of Kanred were returned to the greenhouse after the exposure at −12° C.

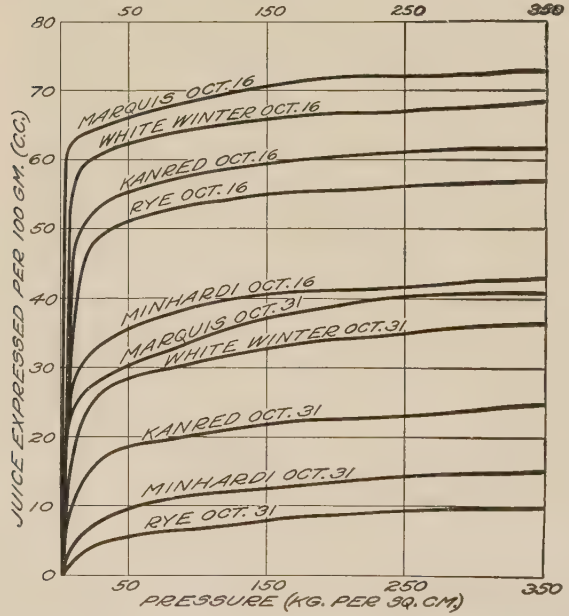

FIG. 4.—Quantities of juice expressed per 100 grams of tissue from frozen leaves of four varieties of wheat and one variety of rye at pressures of 50, 150, 250, and 350 kgm. per square centimeter, on October 16 and October 31, 1925, University Farm, St. Paul, Minn

All of the Kanred plants and half of the Minhardi plants had been killed by this exposure. The subsequent rapid drying was largely

in dead tissue. Under usual freezing conditions most of the leaves are killed. The experiment just described merely demonstrates that even at freezing temperatures drying of wheat leaves may take place rapidly.

It does not seem possible to differentiate between varieties which are rather similar in hardiness by merely determining their moisture contents. Under more favorable conditions for obtaining representative samples, true differences in hardiness might be shown, especially if several samples were taken and the results averaged.

Quantity of Juice Expressed from Leaves

The imbibition pressure of wheat leaves, as measured by the volume of juice or sap expressed from the leaves by given pressures, was found by Newton (*34*, *35*, *36*) to be closely related to hardiness. This test was applied in these studies during both seasons, and in 1923 on both unfrozen leaves and leaves frozen in the hardening room before pressing. In 1925 all samples were frozen before pressing.

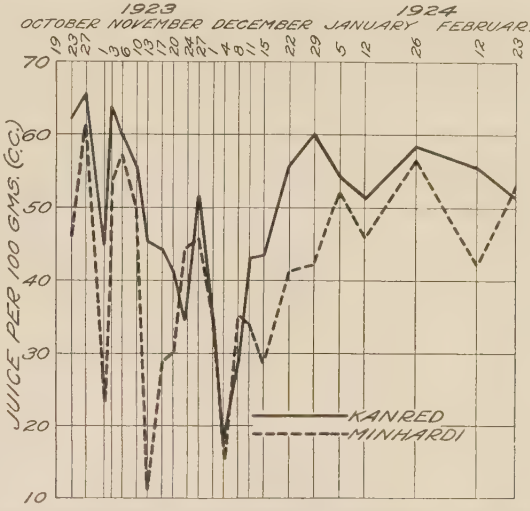

Fig. 5.—Quantities of juice expressed, at a pressure of 339 atmospheres, per 100 grams of frozen leaves of Kanred and Minhard wheats collected at intervals during the autumn and winter of 1923–24, University Farm, St. Paul, Minn.

Although measurements of expressed sap were recorded for pressures of 50, 150, 250, and 350 kgm. per square centimeter, only the quantities expressed at 350 kilograms are presented. All of these measurements, as shown by the curves in Figure 4, showed approximately the same relative values. At 50 kgm., frozen samples of Kanred and Minturki yielded, respectively, 80.6 and 79.5 per cent; at 150 kgm., 91.5 and 92 per cent; and at 250 kgm., 95.4 and 96.5 per cent of the quantity of sap expressed at 350 kgm. It was found by experiment that about 1 c. c. of sap was absorbed on the cloth, bowl, and plunger in pressing, and as the size of sample pressed varied considerably, the data for sap expressed per 100 gm. of material are corrected by adding 1 c. c. to the actual quantity of sap expressed. The pressure readings taken were in kilograms per square centimeter. A pressure of 350. kgm. per square centimeter is equal to 338.7 atmospheres, and approximately the latter measure (339 atmospheres) is used in the tables and textual discussion.

The data for the quantity of juice expressed per 100 gm. of fresh tissue at a pressure of 339 atmospheres, together with the percentage of total moisture which was expressed, are given in Tables 4 and 5 and are shown graphically in Figures 5 and 6.

For unfrozen leaves, the quantity of juice expressed per 100 gm. of unfrozen tissue decreased during the season from 25.4 c. c. for Minhardi and 29.1 c. c. for Kanred on October 19, to 5.2 c. c. for Minhardi and 5.3 c. c. for Kanred on December 8. After the latter date the quantity increased somewhat. A similar relation was shown in the percentages of total moisture expressed, indicating that the decreased quantity of expressed juice was not entirely a measure of the decreased moisture content. More juice was expressed from rye and White Winter wheat than from Kanred and Minhardi wheats, because of their higher moisture content.

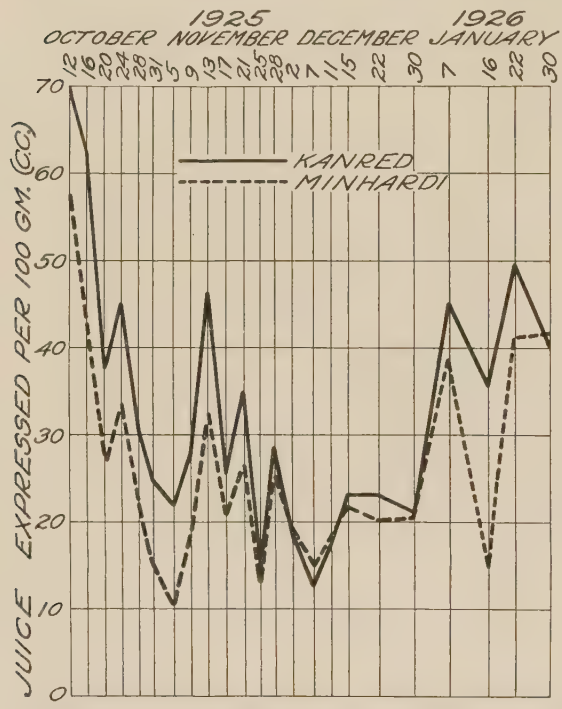

FIG. 6.—Quantities of juice expressed, at a pressure of 339 atmospheres, per 100 grams of frozen leaves of Kanred and Minhardi wheats collected at intervals during the autumn and winter of 1925-26, University Farm, St. Paul, Minn.

The data in Table 4 show that considerably more juice is expressed from the frozen than from the unfrozen tissues. This is due to the increased permeability of the cell membranes and the partial precipitation of the protoplasm as a result of freezing. More juice was expressed from Kanred wheat than from Minhardi and more from White Winter than from Kanred. The quantity of juice expressed from these varieties of wheat previous to the November 20 samplings was always in the inverse order of their hardiness. After that date there were some irregularities, but usually the less hardy varieties yielded more juice than Minhardi. The differences between Kanred and Minhardi in the quantity of juice expressed varied considerably, depending not only upon the moisture content but also upon the effects of freezing upon the tissues. After November 20 the differences were too irregular or too small to be of value in distinguishing between hardy and nonhardy wheats, and after December 4 the quantity of juice obtained from both varieties was too large to be used as a basis for measuring hardiness.

More juice was expressed from rye than from Minhardi wheat until December 1, the date of the last collection of rye. There was little indication from the results obtained in 1923 that rye possessed greater ability to hold water against freezing and pressure than the hardy winter wheats. This may have been partly because the rye was sown nine days later than the wheat. Approximately the same

relationships were shown in percentage of total moisture expressed as in the quantities of juice expressed. The percentage expressed from White Winter wheat was more than 70 per cent except on two occasions, while Minhardi yielded less than 70 per cent of its juice most of the time, except at the beginning and end of the season.

TABLE 4.—*Quantity of juice expressed, at 339 atmospheres pressure, from frozen and unfrozen plants of three varieties of wheat and one variety of rye collected at intervals during the autumn and winter of 1923–24, University Farm, St. Paul, Minn.*

Condition and date collected	Juice per 100 gm. of fresh tissue (c. c.)				Percentage of total moisture expressed			
	Minhardi	Kanred	White Winter	Minnesota No. 2 rye	Minhardi	Kanred	White Winter	Minnesota No. 2 rye
UNFROZEN PLANTS								
October:								
19	25.4	29.1			32.6	35.9		
23	15.4	14.1			20.6	17.8		
27	16.8	20.3			24.1	28.9		
November:								
1	13.9	16.3			20.6	22.0		
3	9.4	12.1	20.3	14.3	12.8	15.7	26.9	18.6
6	9.0	11.3			12.9	15.1		
10	9.9	9.5	14.6	10.1	14.1	13.1	19.9	13.4
13	6.1	8.5			9.0	11.9		
17	5.7	8.3	6.7	7.7	8.1	11.4	9.0	10.3
20	9.0	7.8			12.0	10.6		
24	7.4	8.1	8.1	11.1	10.5	12.1	11.0	16.1
27	6.1	5.6			9.3	7.7		
December:								
1	5.7	5.3	8.4	6.9	7.7	7.2	11.4	9.8
4	5.3	8.5			7.8	11.8		
8	5.2	5.3	6.0		7.6	7.6	8.7	
11[a]	9.7	14.7			13.9	20.9		
15	8.4	10.0	9.2		13.5	14.8	16.5	
22	13.9	7.7	12.4		21.1	10.5	18.4	
29[b]	32.4	35.5	46.5		45.8	47.8	62.2	
Average[c]	10.128 ±0.810	11.25 ±0.927						
FROZEN PLANTS								
October:								
23	46.0	62.1			61.7	79.0		
20	61.3	65.3			88.3	94.1		
November:								
1	23.5	44.9			33.8	61.2		
3	53.6	63.6	68.1	53.4	73.8	83.1	89.1	70.5
6	57.1	59.9			81.8	80.7		
10	50.0	55.4	67.1	52.3	71.2	74.5	93.3	69.9
13	11.3	45.4			16.6	63.9		
10	28.8	44.1	56.8	33.3	40.9	60.6	77.8	45.1
20	30.1	41.3			43.8	55.6		
24	44.4	34.5	51.4	50.1	65.3	50.3	70.6	71.5
27	45.6	51.1			67.2	70.5		
December:								
1	33.4	34.3	36.4	26.4	47.1	46.5	50.0	35.9
4	15.4	18.4			22.2	26.5		
8	35.0	28.2	49.1		52.6	39.3	70.9	
11	34.1	43.1			52.5	62.4		
15	28.5	43.3	35.5		43.0	63.5	60.4	
22	41.4	55.7	58.7		60.5	77.7	87.1	
29	42.2	60.0	58.8		62.4	83.6	86.4	
January:								
5	52.4	54.0	55.3		73.7	76.9	83.2	
12	45.9	51.4	50.3		64.9	75.0	73.3	
26	56.3	58.3	59.5		84.3	82.3	79.2	
February:								
12	42.0	55.3	57.1		62.2	82.6	82.5	
23	53.1	51.1	47.2		81.7	70.6	73.2	
March:								
8	41.0	37.6	27.4		73.8	59.0	42.6	
27	24.7	26.4			40.6	46.2		
May:								
17	68.2	69.4			90.8	88.2		
Average	40.973 ±1.820	48.235 ±1.673	51.913 ±1.854					

[a] Samples partly frozen when gathered.
[b] Samples frozen when gathered.
[c] Not including samples collected Dec. 29.

TABLE 5.—*Average quantity of juice expressed, at 339 atmospheres, from duplicate samples of frozen plants of four varieties of wheat and one variety of rye collected at intervals during the autumn and winter of 1925–26, University Farm, St. Paul, Minn.*

Date collected	Juice expressed per 100 grams (c. c.)					Percentage of total moisture expressed				
	Minhardi	Kanred	White Winter	Marquis	Minnesota No. 2 rye	Minhardi	Kanred	White Winter	Marquis	Minnesota No. 2 rye
October:										
12	57.9	69.3	70.9	69.8	70.9	75.4	88.1	87.3	85.3	88.4
16	43.4	62.1	68.7	73.1	57.2	57.6	80.1	85.3	90.4	73.0
20	26.3	37.4	47.6	56.3	25.4	36.3	50.0	60.8	71.8	32.9
24	33.6	44.9	55.6	64.1	37.4	46.6	59.5	70.2	83.2	50.0
28	21.9	30.9	42.7	41.3	28.7	32.6	42.8	58.6	59.4	39.8
31	15.6	24.9	36.6	40.7	9.7	26.4	39.0	57.4	62.3	15.0
November:										
5	10.5	21.7	34.5	47.2	12.9	15.5	30.7	48.6	65.8	17.8
9	18.6	27.5	34.3	34.0	12.3	31.1	43.5	53.2	59.1	18.8
13	32.1	46.1	45.1	45.8	27.9	48.7	65.2	64.0	69.2	39.2
17	20.8	25.5	30.0	33.7	11.8	32.7	37.4	44.1	55.8	17.3
21	26.5	34.8	42.4	46.7	21.3	40.4	50.2	60.8	68.3	30.4
25	12.9	15.0	19.8	29.3	5.7	20.6	22.1	30.2	29.3	8.0
28	25.4	28.4	32.9	29.6	25.5	41.1	42.5	49.6	47.3	36.2
December:										
2	19.7	19.2	20.0	21.7	6.0	31.8	30.4	32.6	36.7	8.9
7	15.0	12.9	20.6	33.8	7.1	21.8	18.8	31.2	52.2	9.2
11	18.2	17.0	30.7	43.2	9.7	27.3	24.4	44.9	56.6	13.3
15	21.7	23.1	38.1	38.8	14.8	34.5	33.6	58.4	63.4	21.3
22	20.1	23.0	23.4	35.6	25.3	32.2	35.0	37.5	55.5	35.5
30	20.5	21.2	25.8	35.1	18.8	32.9	31.8	44.8	59.6	26.7
January:										
7	38.4	44.9			39.8	55.9	63.6			56.1
16	15.1	35.7	40.8	44.7	10.6	22.4	52.0	62.7	67.1	14.6
22	41.1	49.4			35.1	61.4	70.2			51.2
30	41.8	40.4	57.0	47.8	11.6	56.4	57.5	74.9	79.5	16.8
May:										
4	60.8	64.1			66.0	82.7	82.5			83.3
Average [a]	25.961 ±1.630	32.839 ±2.030	38.929 ±2.121	43.443 ±1.907	22.848 ±2.287	38.3	46.5	55.1	62.8	31.3

[a] Not including samples collected May 4.

In the fall and winter of 1925–26, duplicate frozen samples of four varieties of wheat and one variety of rye were tested for the quantity of juice which could be expressed. (See Table 5.) The quantities of juice expressed from the wheat varieties were in inverse order of their hardiness in most of the collections. In many of the tests, however, the quantity of expressed juice from one or more of the wheat varieties was not in the order of the hardiness of those varieties. At the beginning of the season rye yielded more sap than Minhardi wheat, but after October 28 less juice was expressed from rye than from Minhardi, except on five dates. During part of this period less juice was expressed from rye than from any of the wheats. The small quantity of juice expressed from hardened tissues of Minnesota No. 2 rye, which is hardier than any wheat known, shows that hardiness in cereals is largely a matter of imbibition pressure of the cell colloids and tends further to substantiate the results reported by Newton (*34*, *35*, *36*).

It will be noticed that considerable variation occurs in the quantity of juice expressed, even within short periods. Large differences between collections 15 days apart, during a period in which hardening occurred, are shown in Figure 4. Some of this variation between consecutive collections is due, of course, to experimental and sampling

errors and also to differences in technic or treatment of the samples. Most of the variation, however, can be accounted for either by differences in moisture content or by the differences in the water-holding power of the tissues. This latter characteristic could be determined readily by the appearance of the samples removed from the press bowl. The hardened tissues of hardy varieties were not broken down by freezing and pressure, and when removed from the bowl they were elastic and turgid and expanded when the pressure was released; whereas samples of nonhardy or unhardened tissues remained in a thin compact layer when removed from the press bowl. This difference is shown in Figure 7. It was observed that the samples which yielded most of their juice in pressing showed the characteristics of unhardened samples mentioned above, while samples which yielded very little juice were elastic both before and after pressing. Unhardened samples when thawed were of dark color, flaccid, and watery. On several occasions the samples of Minhardi showed as much injury from freezing as Kanred or more.

The differences which exist between plants in adjoining rows or in successive periods, in the ease with which wheat leaves give up water, partly explain why winterkilling in wheat is so variable under different seasonal and environmental conditions. Adjoining rows of wheat often differ noticeably in winter survival, and winterkilling is often irregular even within a short row. Temperature conditions fatal to wheat in one season may not be injurious in another.

In the winter of 1923–24 the increased quantity of expressed juice during December coincided with a warm period in which the growth of the wheat was resumed and the hardening process reversed. The sudden drop in temperature which followed did not permit hardening to occur again before the plants were permanently frozen. The large quantity of expressed juice obtained during the winter of 1924 probably was due to the abnormal seasonal conditions just described. In December, 1925, a gradually increasing quantity of expressed juice again was obtained, this time during continued cold weather. The increased quantity of expressed juice and the apparent coming out from the hardened condition which occurred in this season might be explained, however, in one or both of two ways. Probably some of the tissues were being killed and broken down, thus losing their power of imbibition. The more probable explanation is the increased effect of gradual freezing in the breaking down of protoplasm, mentioned by Bayliss (*6*). The plants were continuously frozen during December but the extent of freezing was increased by subjecting the tissues to the low temperatures of the hardening room. The increased freezing probably removed additional water from the protoplasm to form ice.

It was thought that some of the variation occurring in the samples might be due to methods of handling. One of the variables was the rate and manner of thawing the material. Usually samples were taken from the freezing room to the laboratory and allowed to thaw at room temperature, but occasionally they were placed on or near the radiator to speed up the process. Slight differences were observed in the quantities of juice expressed from the tissue warmed to room temperature or slightly above, when compared with similar samples which were barely thawed.

Samples of rye were collected December 22, 1925, and were subjected to repeated freezings and thawings for different periods. The

FIG. 7.—Press residues of hardened leaves of wheat and rye plants after being frozen and pressed. Left to right: Kanred, Minhardi, and White Winter wheats, Minnesota No. 2 rye, and Marquis wheat. The hardiness of the varieties is in the direct order of the fluffiness of the samples. Unhardened leaves of the hardy varieties behaved like the Marquis sample

treatments and results shown in Table 6 indicate that thawing to room temperature for a few hours and then freezing again causes only

a slight increase in the quantity of juice expressed. The sample which was frozen and thawed three times yielded only 4.1 c. c. more juice per 100 gm. than the sample thawed but once.

TABLE 6.—*Average quantity of juice expressed, at 339 atmospheres, from duplicate sample plants of Minnesota No. 2 rye collected on December 22, 1925, and subjected to different thawing and freezing periods before pressing, University Farm, St. Paul, Minn.*

Treatment	Date of pressing	Juice per 100 gm.
		C. c.
Thawed and pressed at once	Dec. 29	25.3
Thawed 24 hours at room temperature and pressed	do	28.8
Thawed 6 hours Dec. 29, returned to hardening room, thawed 6 hours, and pressed	Jan. 2	33.9
Thawed at room temperature 6 hours Dec. 29, returned to hardening room; thawed at room temperature 6 hours Jan. 2, returned to hardening room; thawed at room temperature 5 hours and pressed	Jan. 19	29.4

TABLE 7.—*Quantity of juice per gram of dry matter held, after pressing at 339 atmospheres, by frozen and unfrozen plants of three varieties of wheat and one variety of rye collected at intervals during the fall and winter of 1923–24, University Farm, St. Paul, Minn.*

Date collected	Water held per gram of dry matter (grams)							
	Frozen plants				Unfrozen plants			
	Minhardi	Kanred	White Winter	Minnesota No. 2 rye	Minhardi	Kanred	White Winter	Minnesota No. 2 rye
October:								
19					2.36	2.78		
23	1.12	0.77			2.37	3.44		
27	.27	.13			1.76	1.68		
November:								
1	1.52	1.07			1.66	2.22		
3	.69	.55	0.35	0.92	2.44	2.87	2.29	2.70
6	.42	.55			1.99	2.53		
10	.68	.74	.17	.89	2.01	2.28	2.20	2.69
13	1.77	.89			1.90	2.20		
17	1.41	1.06	.60	1.55	2.22	2.39	2.60	2.63
20	1.24	1.28			2.63	2.47		
24	.74	1.09	.79	.67	2.11	1.79	2.52	1.87
27	.69	.78			1.75	2.50		
December:								
1	1.29	1.51	1.34	1.78	2.63	2.56	2.47	2.15
4	1.77	1.67			1.94	2.21		
8	.94	1.54	.66		2.01	2.16	2.02	
11	.88	.84			1.99	1.86		
15	1.12	.78	.57		1.41	1.78	1.05	
22	.85	.57	.27		1.53	2.46	1.70	
29	.78	.42	.40					
January:								
5	.65	.55	.34					
12	.84	.54	.58					
26	.36	.42	.64					
February:								
12	.80	.30	.39					
23	.34	.77	.49					
March:								
8	.34	.72	1.03					
27	.93	.72						
May:								
17	.28	.44						
Average	.874	.796			2.039	2.343		

Duplicate samples of Kanred wheat collected November 4, 1925, during a mist, were left in the laboratory for 20 hours before being placed in the hardening room. These samples had a moisture content only about 2 per cent higher than that of the samples col-

lected the following day and taken to the hardening room immediately, yet they yielded an average of 59.2 c. c. of juice per 100 gm. of tissue, as compared with 21.7 c. c. of juice per 100 gm. of tissue from the latter. Fermentation, which had begun before the first samples were taken to the hardening room, apparently had destroyed the colloidal imbibition of the protoplasm.

A determination of the juice expressed per 100 gm. of fresh tissue does not give an exact measure of the imbibitional capacity of the protoplasm, because the tissue varies in moisture content. The quantity of water held per gram of dry matter after pressing at 339 atmospheres pressure has been calculated, therefore, and the results are presented in Tables 7 and 8. With frozen samples, the placement of varieties or collections is about the same for this relationship as for the quantities of juice expressed per 100 gm. of tissue. On unfrozen samples, however, there is little or no correlation between the quantities of juice held and the hardiness of the varieties. Because of their lower initial moisture content, unfrozen tissues of Minhardi wheat usually held less juice after pressing than those of Kanred.

TABLE 8.—*Average quantity of juice per gram of dry matter held, after pressing at 339 atmospheres, by duplicate samples of frozen plants of four varieties of wheat and one variety of rye collected at intervals during the fall and winter of 1925–26, University Farm, St. Paul, Minn.*

Date collected	Water held per gram of dry matter (grams)				
	Minhardi	Kanred	White Winter	Marquis	Minnesota No. 2 rye
October:					
12	0.81	0.44	0.55	0.66	0.47
16	1.30	.68	.61	.68	.98
20	1.68	1.64	1.42	1.03	2.36
24	1.38	1.25	.96	.56	1.49
28	1.40	1.48	1.12	1.14	1.56
31	1.07	1.09	.77	.71	1.58
November:					
5	1.78	1.68	1.26	.88	2.18
9	1.02	.97	.85	.54	1.54
13	1.01	.85	.86	.60	1.50
17	1.19	1.33	1.20	.68	1.87
21	1.14	1.17	.91	.69	1.62
25	1.35	1.67	1.35	.80	2.27
28	1.08	1.17	.99	.88	1.54
December:					
2	1.11	1.25	1.13	.92	1.91
7	1.70	1.78	1.34	.88	2.31
11	1.46	1.75	1.22	.86	2.30
15	1.10	1.49	.78	.58	2.17
22	1.18	1.27	1.04	.73	1.61
30	1.08	1.37	.75	.57	1.76
January:					
7	.98	.88			1.07
16	1.61	1.07	.70	.66	2.29
22	.80	.70			1.11
30	1.24	1.12	.82	.32	1.88
May:					
4	.48	.61			.64
Average	1.206	1.196			1.667

FREEZING-POINT DEPRESSION OF EXPRESSED JUICE

The freezing-point depression of the expressed juice from the frozen plants of wheat and rye was determined in both years of the investigation. The results for 1925–26 are from duplicate samples. The data are presented in Tables 9 and 10 and are shown graphically in Figures 8 and 9.

TABLE 9.—*Freezing-point depressions of the juice expressed from frozen plants of three varieties of wheat and one variety of rye collected at intervals during the fall and winter of 1923–24, University Farm, St. Paul, Minn.*

Date collected	Freezing-point depression (°C.)			
	Minhardi	Kanred	White Winter	Minnesota No. 2 rye
October:				
23	1.11	1.18		
27	.87	.80		
November:				
1	1.57	1.47		
3	1.67	1.48	1.47	1.48
6	1.69	1.43		
10	1.68	1.51	1.42	1.38
13	.97	1.01		
17	1.32	1.35	1.30	1.26
20	1.35	1.27		
24	1.14	1.03	1.20	1.10
27	1.71	1.49		
December:				
1	1.60	1.30	1.34	1.20
4	1.42	1.32		
8	1.67	1.51	1.56	
11	2.11	2.03		
15	1.86	1.99	1.97	
22	1.34	1.25	1.18	
29	1.77	1.33	1.38	
January:				
5	1.60	1.53	1.55	
26	2.17	1.85	1.70	
March:				
8	2.16	1.26	.60	
May:				
17	1.30	1.15		
Average	1.549 ±0.050	1.388 ±0.042	1.389 ±0.062	

TABLE 10.—*Average freezing-point depressions of the expressed juice from plants of four varieties of wheat and one variety of rye collected at intervals during the fall and winter of 1925–26, University Farm, St. Paul, Minn.*

Date collected	Freezing-point depression (°C.)				
	Minhardi	Kanred	White Winter	Marquis	Minnesota No. 2 rye
October:					
12	1.25	1.15	1.06	0.99	1.11
16	1.17	1.17	1.03	.99	1.16
24	1.36	1.29	1.24	1.11	1.21
28	1.55	1.42	1.40	1.58	1.50
31	2.21	2.19	2.07	2.15	2.10
November:					
5	.98	1.06	1.13	1.02	.91
9	2.13	2.14	2.01	1.83	2.17
13	1.58	1.43	1.46	1.54	1.37
17	1.70	1.72	1.76	1.96	1.71
21	1.57	1.44	1.51	1.58	1.42
25	1.69	1.62	1.86	2.12	1.41
28	1.90	1.80	1.90	2.17	1.77
December:					
2	2.05	1.89	2.03	1.90	1.66
7	1.14	1.18	1.36	1.47	1.13
11	1.21	1.20	1.29	1.03	1.19
15	1.39	1.36	1.36	1.15	1.37
22	1.99	2.03	2.06	1.19	1.55
30	1.88	1.85	1.93	1.33	1.74
Average (first 18 samples)	1.597	1.552	1.581	1.506	1.471
January:					
7	1.45	1.43			1.29
22	1.47	1.33			1.29
May:					
4	1.11	1.04			1.08
Average [a]	1.584 ±0.052	1.535 ±0.051	1.581 ±0.057	1.506 ±0.066	1.453 ±0.048

[a] Not including samples collected May 4.

The juice of the Minhardi variety had an average freezing-point depression greater than that of Kanred and White Winter in 1923-24, but about the same as White Winter in 1925–26. The juice of rye usually had a lower freezing-point depression than any of the wheats. The freezing-point depression, although fluctuating, tended to increase during the autumn, fluctuated during the winter, and then decreased in the spring after growth was well begun. The freezing-point depressions of the wheat varieties obtained during the first three collections of 1925 and also most of the early collections of 1923 were in the direct order of hardiness. Later in the season the more tender varieties were so low in moisture content that the juice was much more concentrated. The freezing-point depression of winter-wheat juice seems to depend upon the moisture content of the tissue, the quantity of soluble materials elaborated, and the quantity of imbibed water. When the plants are hardened, or partly so, and a complete and representative sample of juice can not be obtained by freezing and pressure, a freezing-point depression of the juice obtained is not a measure of the hardiness of a variety of wheat. When hardening or wilting has not occurred, the freezing-point depression of the plant juice is a fairly reliable measure of hardiness in the wheat varieties studied.

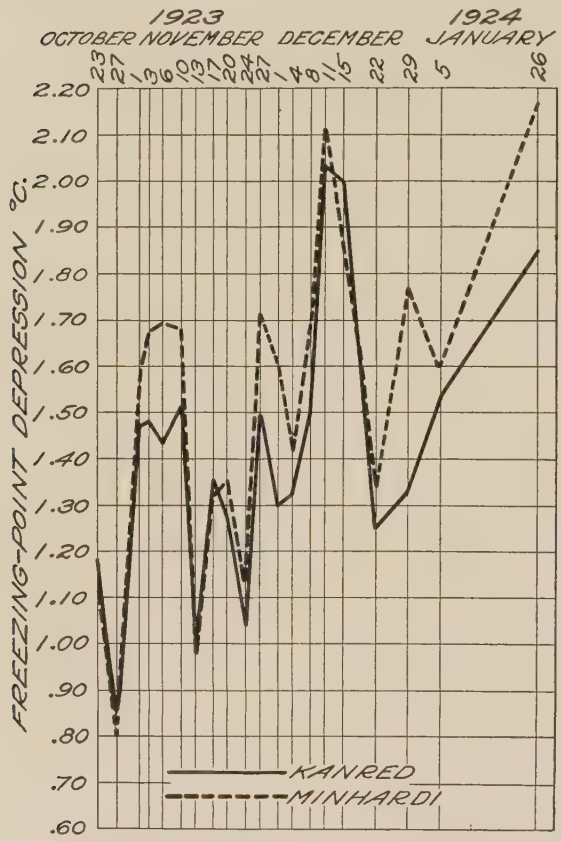

FIG. 8.—Freezing-point depression (°C.) of the juice expressed from the frozen leaves of Kanred and Minhardi wheats collected at intervals during the autumn and winter of 1923–24, University Farm, St. Paul, Minn.

TOTAL SOLIDS IN EXPRESSED JUICE

The percentage of total solids in the expressed juice, as measured by the Abbe refractometer, was determined from both frozen and unfrozen samples in 1923–24. The results are given in Table 11 and are shown graphically in Figure 10. Higher percentages of solids nearly always were present in the juice from frozen plants. Considerable variation existed in the juice from plants collected on different dates, due to variations both in juice concentration and in

the quantities of juice expressed. The percentage of total solids in the juice was higher in Minhardi than in Kanred, and higher in Kanred than in White Winter. The hardier the variety the higher was the content of total solids in the juice on most dates and as an average. Rye juice usually had a lower content of total solids than that of any of the wheats. The total solids determined consisted of material in both molecular and colloidal solution, but probably mostly the former. The higher percentage of total solids in hardy varieties probably is due largely to the lower moisture content, and the increase in total solids during hardening both to decreased moisture content and to the increase in soluble carbohydrates and nitrogen compounds. The higher percentage of total solids in the frozen tissue shows that the disorganization of the protoplasm by freezing causes a release of soluble material not obtained by pressure without freezing. The juice obtained from the unfrozen tissue probably came largely from the vacuoles.

TABLE 11.—*Total solids in the juice, expressed at 339 atmospheres pressure, from unfrozen and frozen plants of three varieties of wheat and one variety of rye collected at intervals during the fall and winter of 1923–24, University Farm, St. Paul, Minn.*

Date collected	Total solids (per cent)							
	Unfrozen samples				Frozen samples			
	Minhardi	Kanred	White Winter	Minnesota No. 2 rye	Minhardi	Kanred	White winter	Minnesota No. 2 rye
October:								
19	8.8	9.3						
23	13.6	11.6			11.2	10.0		
27[a]	3.6	2.7			9.0	7.0		
November:								
1	13.5	8.0			18.0	17.5		
3	13.0	11.0	9.0	10.0	15.0	13.8	12.3	13.5
6	10.5	8.5			16.5	14.0		
10	10.5	10.5	11.0	11.0	16.5	14.3	13.8	13.5
13	9.5	10.0			11.0	10.5		
17	12.0	10.4	12.5	10.0	12.5	12.3	11.2	11.3
20	11.0	11.0			14.5	13.3		
24	8.0	9.5	8.3	8.0	12.0	10.0	11.0	11.0
27	9.5	10.5			16.5	14.5		
December:								
1	9.8	7.0	7.5	5.4	13.0	10.0	10.0	8.0
4	10.0	10.0			10.5	9.0		
8	12.7	11.3	10.0		18.0	14.0	15.0	
11	15.5	13.0			19.5	18.5		
15	19.5	16.5	15.0		18.0	20.0	18.0	
22	14.0	14.0	8.0		13.0	12.0	11.5	
29					15.5	10.0	13.0	
January:								
5					12.3	11.0	12.1	
12					14.8	11.0	10.5	
26					16.5	11.8	11.6	
February:								
12					12.5	12.9	6.5	
26					10.7	8.5	11.5	
March:								
8					18.0	10.0	6.0	
27					11.0	9.5		
May:								
17					4.2	6.3		
Average	11.389 ±0.524	10.267 ±0.447			13.854 ±0.460	11.988 ±0.426	11.600 ±0.496	

[a] Samples gathered on Oct. 27 contained excess moisture due to snow.

Bound Water in Expressed Juice

The determination of bound water was made in 1923–24 on juice from frozen samples only. The average percentage of bound water in the juice of Minhardi, Kanred, and White Winter varieties varied directly with hardiness. Rye juice showed a bound-water content greater than that of wheat on some dates and less on others. There was extreme irregularity in the quantities of bound water present. The quantity depends both on the binding power of the colloids in the cells and on the quantity of colloidal material which is removed in expressing the juice. Probably the effect of freezing on the precipitation and dispersion of the colloids also has some effect. The plant tissue was not macerated before pressing, so it is unlikely that the colloids in the juice represent the colloidal conditions within the cells. The fluctuations in the percentage of bound water in the juice obtained by the method used render this test of little value in measuring hardiness. The data on bound water are given in Table 12 and are shown graphically in Figure 11.

Table 12.—*Bound water in the juice expressed from the frozen plants of three varieties of wheat and one variety of rye collected at intervals during the autumn and winter of 1923–24, University Farm, St. Paul, Minn.*

Date collected	Bound water (per cent)			
	Minhardi	Kanred	White Winter	Minnesota No. 2 rye
October:				
23	5.8	7.8		
27	6.5	10.0		
November:				
1	0	1.1		
3			1.9	
10	9.7	10.1	8.0	13.9
13		4.7		
17	16.6	7.3	11.1	5.0
20	15.4	13.3		
24	8.3	4.3	2.7	8.0
27	6.5	8.0		
December:				
1	4.7	12.3	5.0	10.4
4	7.3	7.3		
8	17.1	9.4	6.9	
11	14.2	5.0		
15	3.5	1.5	6.9	
22	3.5	2.3	5.0	
29	8.0	10.4	6.9	
January:				
5	3.9	9.7	6.5	
26	7.3	8.0		
March:				
8	16.3	9.0		
May:				
17	11.9	11.6		
Average	8.763 ±0.768	7.655 ±0.516	6.090 ±0.532	

Correlations Between Characters Used to Measure Hardiness

In order to determine the relation of the results obtained in the various tests on the wheat and rye samples, correlation coefficients were calculated. The variables used were from the average of duplicate determinations made in 1925–26 and from single determinations made in 1923–24, except that the moisture-content determinations of

this season also were in duplicate. Data obtained during the entire season were used, thus including data from both hardened and non-hardened plants. The data used in calculating the coefficients were from 15 to 26 experiments in each case, except those on White Winter wheat in 1923–24, of which only 8 to 12 items were available. The correlation coefficients were computed according to the usual formula when using ungrouped variables:

$$r = \frac{\frac{\Sigma x y}{n} - \left(\frac{\Sigma x}{n}\right)\left(\frac{\Sigma y}{n}\right)}{\sqrt{\frac{\Sigma x^2}{n} - \left(\frac{\Sigma x}{n}\right)^2} \times \sqrt{\frac{\Sigma y^2}{n} - \left(\frac{\Sigma y}{n}\right)^2}}$$

when Σ = summation, x = values of one variable, y = values of the other variable, and n = number of observations.

The correlation coefficients obtained are given in Table 13. The quantity of juice expressed is positively correlated, in most cases highly so, with the moisture content of both frozen and unfrozen plants. It is negatively correlated in most cases with freezing-point depression and percentage of total solids in the juice. The freezing-point depression shows a high positive correlation with the percentage of total solids and, especially in 1925–26, a high negative correlation with moisture content. The percentage of total solids is negatively correlated with moisture content. The percentage of bound water does not show any consistent correlations with the other factors measured, the coefficients with Kanred even differing in sign from those of the other two wheat varieties.

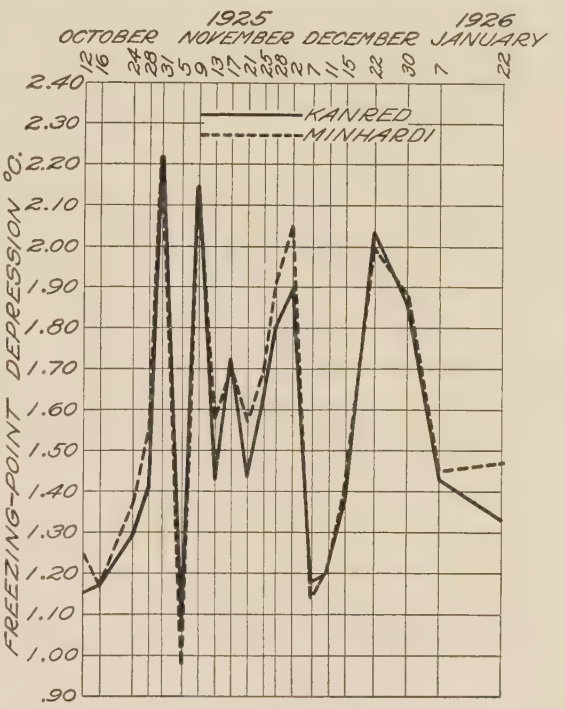

FIG. 9.—Freezing-point depression (°C.) of the juice expressed from the frozen leaves of Kanred and Minhardi wheats collected at interval, during the autumn and winter of 1925–26, University Farm, St. Paul, Minn.

The quantity of juice expressed per 100 gm. of tissue is not an entirely comparable measure when the varieties differ in moisture content. Correlations between the quantity of juice held against freezing and pressure and the moisture content were calculated, therefore, and the coefficients shown in Table 14 were obtained.

TABLE 13.—*Correlation coefficients between physical factors of plants of four varieties of wheat and one variety of rye collected at intervals during the autumns and winters of 1923–24 and 1925–26, University Farm, St. Paul, Minn.*

Factor and variety	Correlation coefficients (*r*) between—			
	Juice expressed (cubic centimeters per 100 gm.)	Freezing point depression ° C.	Percentage of total solids	Percentage of bound water
Frozen samples, 1923–24				
Moisture content (per cent):				
Minhardi	+0.313±0.119	−0.462±0.113	−0.351±0.116	−0.168±0.150
Kanred	+ .533± .095	− .304± .131	− .008± .132	+ .274± .140
White Winter	+ .654± .100	− .094± .193	− .205± .167	− .192± .205
Juice expressed (cubic centimeters per 100 grams):				
Minhardi		+ .079± .143	− .204± .127	− .017± .155
Kanred		− .102± .142	− .107± .131	+ .151± .147
White Winter		− .405± .163	+ .169± .169	− .045± .213
Freezing-point depression (° C.):				
Minhardi			+ .754± .062	+ .166± .150
Kanred			+ .782± .056	− .276± .139
White Winter			+ .873± .046	+ .160± .208
Total solids (per cent):				
Minhardi				+ .094± .153
Kanred				− .533± .108
White Winter				+ .225± .202
Unfrozen samples, 1923–24				
Moisture content (per cent):				
Minhardi	+ .561± .109		− .336± .141	
Kanred	+ .477± .123		− .110± .156	
White Winter	+ .280± .220		− .634± .143	
Juice expressed (cubic centimeters per 100 grams):				
Minhardi			− .160± .155	
Kanred			− .312± .144	
White Winter			− .191± .230	
Frozen samples, 1925–26				
Moisture content (per cent):				
Rye	+ .717± .068	− .757± .064		
Minhardi	+ .696± .073	− .827± .049		
Kanred	+ .734± .065	− .805± .053		
White Winter	+ .868± .036	− .674± .087		
Marquis	+ .897± .029	− .624± .097		
Juice expressed (cubic centimeters per 100 grams):				
Rye		− .388± .128		
Minhardi		− .310± .136		
Kanred		− .387± .128		
White Winter		− .811± .054		
Marquis		− .670± .088		

TABLE 14.—*Calculated correlation coefficients between the quantity of juice held against freezing and pressure and moisture content of plants of Kanred and Minhardi wheats*

Variety	Correlation coefficients (*r*)	
	Frozen samples	Unfrozen samples
Kanred	0.088±0.131	0.898±0.030
Minhardi	.112± .131	.916± .026

While the correlation between moisture content and juice held against expression is positive, it is too small in the frozen samples to be significant. With the unfrozen samples, on the other hand, the correlation coefficients of 0.898 and 0.916 indicate that the moisture content probably is the chief factor in determining how much juice can be expressed from the tissues. If the tissues are sufficiently wilted, no juice can be expressed from the samples at the pressures used.

The correlations between the quantity of juice expressed and the moisture content of the frozen samples in 1925–26 are much higher than in 1923–24. It will be observed that the values of the correlation coefficients in the wheats in both seasons are in the inverse order of hardiness. The regression coefficients for the samples of 1925–26 were calculated, and the regression lines for the moisture contents for given quantities of juice expressed are shown in Figure 12. By extrapolating the regression lines somewhat beyond the limits of the data, the approximate moisture content at which no juice could be expressed at 339 atmospheres pressure after freezing is shown. These theoretical moisture contents at which sap can not be expressed are in the direct order of the hardiness of the varieties, except that Kanred and Minhardi are reversed. This shift is the result partly of an occasional larger juice yield from Minhardi than from Kanred late in the season, but chiefly of the lower average moisture content of Minhardi and its ability to thrive at a considerably lower moisture content.

FIG. 10.—Percentage of total solids in the juice expressed from frozen and unfrozen leaves of Kanred and Minhardi wheats collected at intervals during the autumn and winter of 1923-24, at University Farm, St. Paul, Minn.

Partial correlation coefficients were calculated on some of the factors, to compensate for the variation in the moisture content of the samples. These coefficients are given in Table 15. The partial correlation coefficients between juice expressed per 100 gm. of tissue and freezing-point depression are not significantly different from the gross correlation coefficients for 1923–24. Slight positive but not significant partial correlations between juice expressed and bound water were obtained in all three varieties; the higher the correlation the less hardy is the variety. It is still apparent that bound water in

the juice expressed from unground frozen tissue has little direct relation to imbibition pressure in the tissues, but the trend of the partial correlation coefficients indicates that the more the tissue is broken down in freezing and pressing the more colloidal material the juice will contain.

TABLE 15.—*The gross correlation coefficients for juice expressed per 100 gm. of tissue with freezing-point depression and bound water and the partial correlation coefficients (assuming that the moisture content is held constant) obtained on wheat and rye in 1923–24 and 1925–26*

Season and variety	Correlation coefficients for juice expressed per 100 grams of tissue			
	Gross correlation coefficients		Partial correlation coefficients [a] (moisture content constant)	
	Freezing-point depression (r_{23})	Bound water (r_{24})	Freezing-point depression ($r_{23.1}$)	Bound water ($r_{24.1}$)
Season of 1923-24:				
Minhardi	+0.079	−0.017	+0.265	+0.004
Kanred	− .102	+ .151	+ .198	+ .001
White Winter	− .405	− .045	− .456	+ .109
Season of 1925–26:				
Rye	− .388			+ .340
Minhardi	− .310			+ .641
Kanred	− .387			+ .506
White Winter	− .811			− .616
Marquis	− .670			− .319

[a] Partial correlation coefficients were computed by the formula: $r_{23.1}=\frac{r_{23}-(r_{12}\times r_{13})}{\sqrt{1-r^2 12}\quad\sqrt{1-r^2 13}}$

The nonhardy varieties, Marquis and White Winter, had much higher gross correlations between juice expressed and freezing-point depression than the more hardy varieties, Minhardi, Kanred, and Minnesota No. 2 rye. This showed that the quantity of juice expressed from the less hardy varieties depended more upon the moisture content than in the case of the hardy varieties, and that the lower the moisture content the greater was the freezing-point depression. The hardy varieties, being able to harden, did not yield their juice so completely, as a rule, and thus the concentration or freezing-point depression of the juice varied both with the moisture content of the tissue and in the extent to which the tissue was broken down in freezing and pressing. The partial correlations, assuming moisture content to be constant, still show the freezing-point depression of the juice of the two nonhardy varieties, Marquis and White Winter, to

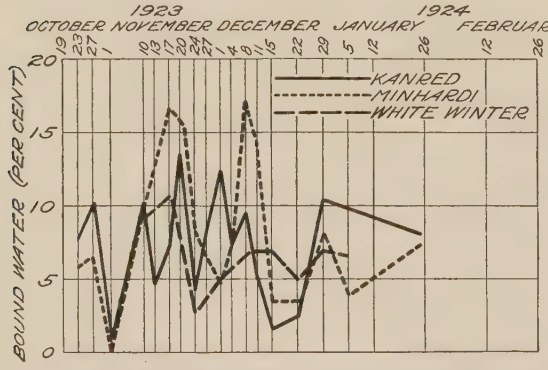

FIG. 11.—Percentage of bound water in the juice expressed from frozen leaves of Kanred, Minhardi, and White Winter wheats collected at intervals during the autumn and winter of 1923-24, University Farm, St. Paul, Minn.

be negatively correlated with the quantity of juice expressed, while in the juice of the three hardy varieties, Minhardi, Kanred, and Minnesota No. 2 rye, these two factors are positively correlated. These results may be merely chance differences, as such relationships were not obtained in the 1923–24 samples. On the other hand, they may indicate that hardy varieties usually yield the more dilute vacuolar sap, but as the protoplasm is broken down, juice which contains more dissolved material is expressed. The nonhardy varieties are almost completely broken down by the freezing at all times, and it appears that the more completely the juice is expressed the more dilute it becomes.

Rate of Respiration at Low Temperatures

The results obtained by Govorov (*18*) indicated that hardy wheats and ryes respired less than nonhardy varieties at low temperatures. Newton (*36*) found that hardy varieties retained their sugars throughout the winter more completely than the nonhardy varieties. Experiments were undertaken by the writer to determine whether these sugars were used up as the result of a greater rate of respiration. Plants of wheat and rye were grown in glazed pots in the greenhouse. Before starting a pot of plants in the respiration experiment, the soil was sealed over with a half-and-half mixture of vaseline and liquid paraffin, which, after melting, could be poured at a temperature of 40° to 45° C. on the soil and around the bases of the plants. The cover was then placed over the plants with its edge resting on the seal inside the pot. The space between the cover and the pot then was sealed. The pots were first placed in the chamber at 5° C., and when the rate of respiration had been determined they were transferred successively to other chambers with temperatures of 0°, −5°, and −10° C., respectively. After the pots had come to a temperature equilibrium in a chamber, 8 to 12 hours being allowed, the CO_2 present was removed with a stream of air from which the CO_2 had been removed, and the plants were alowed to respire. The gradual reduction in temperature permitted the winter varieties to survive the final temperature of −10° C., but the spring wheat Marquis did not survive. The winter varieties showed growth after passing through the respiration experiments and being cut off just above the wax seal. This indicated that the seal used was not toxic. The data obtained in the respiration experiment are presented in Table 16.

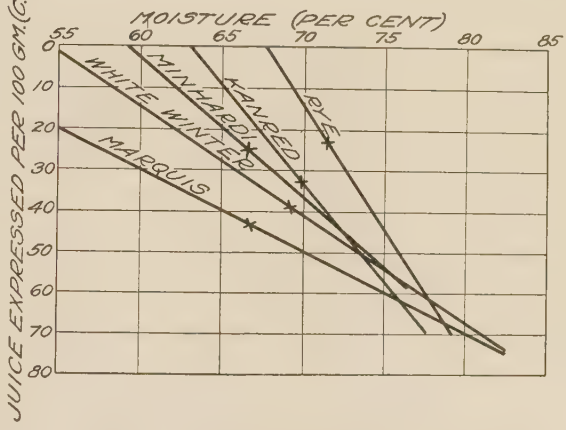

Fig. 12.—Regression lines for the mean values of moisture content plotted against the quantity of juice expressed per 100 gm. of frozen leaves of four varieties of wheat and one variety of rye

TABLE 16.—*Quantity of CO_2 respired by plants of four varieties of wheat and one variety of rye placed successively in chambers at four different temperatures*

Variety and sample		Milligrams of CO_2 respired per hour per kilogram of dry matter at—				Van't-Hoff coefficient −10° to 0° C.
		5° C.	0° C.	−5° C.	−10° C.	
Minhardi	1	126.4	313.0	221.2	66.3	4.7
	2	320.5	142.2	163.0	60.8	2.3
	3	435.0	322.6	120.9	65.7	4.9
Average		294.0	259.3	168.4	64.3	4.0
Kanred	1	351.5	299.5	141.4	88.3	3.5
	2	323.9	270.6	243.3	185.5	1.4
	3	258.7	200.2	181.2	92.1	2.2
Average		311.4	256.8	188.6	122.0	2.4
Red Rock	1	473.5	306.7	219.3	217.1	1.4
	2	150.7	37.7	31.3	21.9	1.7
	3	357.4	203.2	173.4	49.8	4.1
Average		327.2	182.5	141.3	96.3	2.4
Marquis	1	514.9	261.6	143.3	53.0	4.9
	2	475.0	316.5	225.0	53.9	5.9
	3	325.5	143.0	193.8	44.0	3.3
Average		438.5	240.4	187.4	50.3	4.7
Rye	1	672.7	689.6	106.5	30.1	22.9
	2	359.5	433.0	158.5	58.2	7.4
	3	442.8	428.2	232.4	124.0	3.5
Average		491.7	516.9	165.8	70.8	11.3

At the higher temperatures, 5° and 0° C., rye showed the greatest rate of respiration. Marquis, a spring wheat, showed the second greatest rate of respiration at 5°. The low respiration of Minhardi in the first experiment at 5° C. can not be explained. A plant adjustment may have been taking place, or possibly the temperature or air-circulation controls were temporarily out of order and this was not detected. The latter possibility does not seem likely, however, as the quantity of CO_2 respired by the Minhardi plants at 5° C. agreed very closely during two successive periods, indicating an actual depression in respiration.

At the low temperature of −10° C. the quantity of CO_2 obtained per kilogram of dry matter in the winter varieties was in the inverse order of hardiness. The low rate of respiration of Marquis at −10° C. probably was due to the plants having been killed while the winter wheats and rye were not. The Van't-Hoff coefficients calculated for the range of −10° C. to 0° C. show that the increase in respiration between these temperatures was in the order of hardiness of the winter grains. The results, especially those with rye, indicate that hardy varieties are able to conserve their carbohydrates at low temperatures to a much greater extent than are the nonhardy.

DETERMINATION OF LETHAL TEMPERATURES FOR WHEAT

The accidental killing of two of the four varieties of wheat growing in pots in the greenhouse when they were placed outside to harden showed that varieties differed in their ability to withstand low temperatures without previous hardening. Experiments were then begun

to determine the lethal temperatures of wheat and rye taken directly from the greenhouse and also to determine the protection afforded by previous exposure to different low temperatures before subjecting them to temperatures likely to kill. The results obtained are shown in Table 17.

The tests in 1924 were made in the large refrigeration chamber belonging to the division of entomology of the University of Minnesota and those in 1926 were made in the ice-cream cabinet used for the respiration experiments.

TABLE 17.—*Rye and wheat plants killed by exposure to various temperatures during various periods in 1924 and 1926*

Temperatures (° C.) and duration of exposure	Plants killed (per cent)										
	Minnesota No. 2 rye	Minhardi	Kanred	Fulcaster	Dawson	Red Rock	White Winter	Marquis	Kubanka	Hard Federation	Kanred×Minhardi, F_2 hybrids
RESULTS IN 1924											
−4° 24 hours								0			
−8° 24 hours	0	0	0				96	100			
−10° 24 hours	100	100	100				100				
−12° 24 hours		100	100								
−12° to −15° 24 hours	100	100	100								
−15° 24 hours	100	100	100								
1° 72 hours, −8° 36 hours, −12° 12 hours		50	100								55.3
1° 72 hours, −8° 36 hours, −9° 12 hours		18	6				100				62.6
3° to 6° one month, −10° 24 hours							100				
3° to 6° one month, −15° 24 hours	100	100	100								
RESULTS IN 1926											
−10° 48 hours	100					100					
−7° 24 hours	0		0	50		0	100	100	100		
−5° 24 hours			5				0	100	100	100	
−5° 24 hours (old plants)								0			
−4° 24 hours			0				0	0	0	0	
5° 48 hours, −10° 24 hours		50									
0° 48 hours, −10° 24 hours		20									
−5° 48 hours, −10° 24 hours		80									
0° 48 hours, −5° 24 hours, −7° 48 hours	20	0	39	100	100						
−7° 24 hours (wilted plants)	0		0								
−7° 24 hours (not wilted)	100		100								

All varieties of wheat and rye tested were killed by an exposure for 24 hours to temperatures of −10° C. or lower after being taken directly from the greenhouse. The Minnesota No. 2 rye and the Minhardi and Kanred wheats completely survived temperatures of −8° C. for 24 hours, while 96 per cent of the White Winter and all of the Marquis plants were killed. At −7° C. for 24 hours the rye, Kanred wheat, and one pot of Red Rock wheat survived completely. One lot of Fulcaster was completely killed at the −7° C. temperature, but another lot completely survived although showing severe injury. White Winter, Marquis, and Kubanka were completely killed at −7° C. At −5° C., Kubanka and Hard Federation spring wheats were completely killed. Two lots of Marquis also were

completely killed at this temperature, although one pot containing older plants survived completely at this temperature. White Winter completely survived a temperature of −5° C., as also did all lots of Kanred but one, which showed some injury. Plants of Marquis, which were starting to head, completely survived a temperature of −4° C. for 24 hours. Plants of Marquis, Hard Federation, Kubanka, White Winter, and Kanred, which had not quite reached the jointing stage, completely survived a temperature of −4° C. for 24 hours.

In tests with Minhardi wheat at 0° C. for 48 hours, 80 per cent survived, while at −5° C. for 48 hours only 20 per cent of the plants survived. A previous exposure to 5° C. for 48 hours enabled half of the plants to survive −10° C. for 24 hours. In tests in which the temperatures successively were 0° C. for 48 hours, −5° C. for 24 hours, and −7° C. for 48 hours, 20 per cent of the rye, 39 per cent of the Kanred, and all of the Fulcaster and Dawson, but none of the Minhardi plants, were killed. In other tests, in which the temperatures used were 1° C. for 72 hours, −8° C. for 36 hours, and −12° C. for 12 hours, half of the Minhardi and all of the Kanred plants were killed, while with temperatures of 1° C. for 72 hours, −8° C. for 36 hours, and −9° C. for 12 hours, 18 per cent of the Minhardi plants, 6 per cent of the Kanred plants, and all of the White Winter plants were killed. Plants of White Winter kept for about a month at 3° to 6° C. could not withstand −10° C. for 24 hours, nor could rye, Minhardi, and Kanred, kept under the same conditions, survive −15° C. for 24 hours.

No consistent differences could be observed in the hardiness of wheat seedlings grown in the greenhouse and varying in age from 6 weeks to 4 months.

Plants of Minnesota No. 2 rye and Kanred wheat, which were severely wilted owing to a lack of soil moisture, recovered completely from a freezing of −7° C., while unwilted plants of the same varieties liberally watered were completely killed by this temperature.

HARDINESS OF PLANT PARTS

Plant parts differ in their ability to withstand freezing or desiccation, as shown by Schander and Schaffnit (*50*), Schaffnit (*48*), and Chandler (*9*). When wheat plants are frozen the tips of the oldest leaves show the first injury and wilting, but at extremely low temperatures the entire leaf is wilted. Injury progresses in a given plant from the older to the younger leaves, upon exposure to successive intensities of cold. The same effect occurs in a series of plants each held at one of a series of constant low temperatures. The crown containing the meristematic tissues is the most hardy part of the wheat plant, at least of the portion above the soil surface. Under severe winter conditions all of the wheat leaves die, but if the crown is alive recovery takes place.

In order to determine whether or not all parts of the plants had similar physicochemical properties, different portions of the plants were tested separately on one date in 1923 for moisture content, expressed juice, total solids in the juice, freezing-point depression of the juice, and bound water in the juice, and on two dates in 1925 for moisture content, expressed juice, and freezing-point depression. The results of these determinations are given in Table 18.

TABLE 18.—*Moisture content, juice expressed, total solids in the juice, freezing-point depression of the juice, and bound water in the juice obtained from plant parts of wheat and rye in 1923 and 1925, University Farm, St. Paul, Minn.*

Variety, date of collection, and plant parts	Moisture	Juice expressed per 100 grams at 339 atmospheres	Total solids in juice	Freezing-point depression	Bound water
Minhardi wheat, Dec. 1, 1923:	*Per cent*	*C. c.*	*Per cent*	*° C.*	*Per cent*
Leaves, unfrozen	74.0	5.7	9.8		
Roots and crown, unfrozen	67.2	13.4	6.5		
Leaves, frozen	70.9	33.4	13.0	1.60	4.7
Roots and crown, frozen	65.1	29.9	14.0	1.48	15.7
White Winter wheat, Dec. 1, 1923:					
Leaves, unfrozen	73.6	11.4	7.5		
Roots and crown, unfrozen	67.8	21.1	6.0		
Leaves, frozen	72.8	50.0	10.0	1.34	5.0
Roots and crown, frozen	68.7	37.0	9.0	1.09	12.3
Kanred wheat, Nov. 5, 1925:					
Crown, frozen	70.9	53.5		1.56	
Young leaves, frozen	72.9	55.9		1.59	
Old leaves, frozen	71.3	59.4		1.52	
Minn. No. 2 rye, Nov. 18, 1925:					
Roots, frozen	[a] 79.3	55.9		.58	
Crown, frozen	70.6	26.2		1.44	
Young leaves, frozen	69.8	45.7		1.50	
Old leaves, frozen	70.0	45.3		1.54	

[a] Some wash water on roots, which accounts for higher moisture content, more juice expressed, and lower freezing-point depression.

The frozen samples of the crown and roots of Minhardi and White Winter wheats collected December 1, 1923, contained slightly less moisture than the leaves and yielded correspondingly less juice. The total solids were about the same, and the freezing-point depression of the juice from the crown and roots was about the same as those of the leaves. The chief difference in the two plant portions was in the percentage of bound water. More than three times as much bound water was found in the juice of the crowns and roots as in the leaves of Minhardi, and nearly two and one half times as much in the case of White Winter. Tests for moisture content, quantity of juice expressed, and freezing-point depression, made on the crowns, young leaves, and old leaves of Kanred wheat collected November 5, 1925, yielded very similar results. None of the tissues showed any indication of being especially hardened at this time, although Kanred leaves collected in the forenoon of the same day and left in the laboratory a shorter time yielded less than half as much juice. This indicates that hardening takes place throughout the living portion of the plant and that changes can take place very quickly. The samples of rye collected on November 18, 1925, showed that the crown yielded less juice and consequently was the hardiest portion of the plant. Accurate determinations could not be made on the roots because of the necessity of washing the dirt from the roots with water.

HARDINESS IN WHEAT HYBRIDS

FIELD EXPERIMENTS

The inheritance of hardiness in wheat has been studied by Nilsson-Ehle (*39*) and by Hayes and Garber (*20*). Hybrids were obtained which were hardier than either parent, but the genetic factors involved in hardiness were not determined. Gaines, according to

Schafer (*47*),[6] reported a recessiveness of hardiness in crosses between Turkey (winter) and Jenkin (spring Club) wheats.

The survival of some winter-wheat hybrids in comparison with the parent varieties, the protection of winter wheat obtained by sowing in stubble, and some of the difficulties encountered in determining the hardiness of hybrids in the field will now be considered. Most of these data were obtained in connection with breeding experiments of the western wheat investigations project of the Office of Cereal Crops and Diseases, which were carried on cooperatively at several western field stations. Some results, however, were secured at University Farm, St. Paul, Minn.

The bulked progenies of 36 different crosses, involving combinations of 16 hardy winter-wheat varieties, were grown in the F_3 generation at Dickinson, N. Dak., in 1922 on both fallow and stubble land. Most of the wheat hybrids and parent varieties sown on fallow land were completely winterkilled, and only six crosses showed more than 1 per cent survival. On stubble land the survival of the hybrids ranged from 3 to 100 per cent. The hybrids were mostly intermediate between the parents in their survival, but a few showed survivals approaching the less hardy or the more hardy parent.

In the field the survival of three crosses was observed in detail in the F_3 generation from the progenies of F_2 plants. The cross Kanred × Minhardi was grown at Dickinson, N. Dak., in 1923 and at St. Paul, Minn., in 1926; the Buffum × Eureka cross was grown at Fargo, N. Dak., in 1922 and at Moccasin, Mont., in 1923; and the Minturki × Turkey cross was grown at Dickinson, N. Dak., in 1923. Attempts to study several other crosses were prevented because of either complete killing or of no killing. The number of hybrid lines varied from 99 to 257 in the different tests, in comparison with 11 to 50 parental rows. The hybrid material was all grown under conditions free from winterkilling in the F_1 and F_2 generations. The Kanred × Minhardi hybrids showed survivals approximately intermediate between the parents. The Buffum × Eureka hybrids showed a survival similar to that of the more hardy Buffum parent under the mild conditions at Fargo, N. Dak., in 1922, but under more severe conditions at Moccasin, Mont., in 1923, they were killed as badly as the less hardy Eureka parent.

The Minturki × Turkey hybrid lines survived about as well as the average of the parent varieties. The genetic factors for winter hardiness could not be determined in any of the crosses studied. Much difficulty was encountered in attempting to determine the survival of hybrids in the field, because of the irregularity of winterkilling and the frequency of complete survival or complete killing in several localities.

ARTIFICIAL FREEZING EXPERIMENTS

Artificial freezing was attempted at University Farm, St. Paul, Minn., in order to determine the inheritance of hardiness. Plants were grown in pots in the greenhouse, subjected to low temperatures in a refrigerator, and then returned to the greenhouse and their recovery noted. The results are shown in Table 17.

[6] These inheritance studies were made by E. F. Gaines, cerealist of the Washington Agricultural Experiment Station, in cooperation with the Office of Cereal Crops and Diseases of the United States Department of Agriculture.

In the test involving the series of temperatures, 1° C. for 72 hours, −8° C. for 36 hours, and −12° C. for 12 hours, in which all of the Kanred plants and half of the Minhardi plants were killed, 26 out of 47 or 55.3 per cent, of the F_2 hybrid plants of the cross between these two varieties were killed. This indicated a dominance of hardiness. In the other experiment, under somewhat less severe conditions with successive temperatures of 1° C. for 72 hours, −8° C. for 36 hours, and −9° C. for 12 hours, 62.6 per cent of a total population of 369 F_2 plants were killed. In this test, however, Minhardi was injured somewhat more than Kanred, so the significance of this survival could not be determined.

The only facts regarding the nature of inheritance of hardiness brought out by all of these results are that most of the F_3 strains tend to be intermediate to the parents in hardiness and that the hardier the parents used in the cross are the greater will be the average hardiness of the hybrids. The F_3 and later progenies may show all degrees of survival varying from that of one parent to that of the other, with possibly some transgressive inheritance, as indicated by strains being more or less hardy than either parent. Hardiness in wheat varieties seems to differ over a wide and continuous range. Many varieties doubtless differ genetically in one or more of the factors determining hardiness. It is difficult to find conditions under which one of two varieties of wheat of the same class kills entirely and the other completely survives. Usually both varieties are partly killed, or completely killed, or both survive completely. The extent to which the parents are killed seems largely to determine the extent of killing in their hybrid progeny.

MOISTURE CONTENT OF HYBRIDS

After observing that the difference in moisture content between Kanred and Minhardi wheats existed under nearly all conditions and that moisture content appeared to be related to winter hardiness, it seemed feasible to determine how the moisture difference was inherited.

The material selected for this experiment consisted of the progeny from each of 152 F_2 plants from a Kanred × Minhardi cross grown at University Farm in 1924 in 5-foot rows in conjunction with the parent varieties which occupied adjoining rows distributed at intervals of every eight hybrid rows.

The number of samples which could be gathered, weighed, and dried in the oven within a given period was limited, so that moisture determinations begun on June 3, 1924, were not completed until June 14. Rains occurred during this period, and the moisture content of the samples gathered near its close was slightly higher than that of those gathered at the beginning. The results are shown in Table 19.

The Kanred wheat had an average moisture content of 79.12 ± 0.18 per cent. The Minhardi averaged 76.91 ± 0.21 per cent moisture. The moisture content of the hybrids extended over a range practically the same as that of the two parents combined. The number of rows of both parents and hybrids, however, was not sufficient to give particular significance to the range of variability. The hybrids show a fairly close approximation to a normal frequency distribution when grouped into classes differing in moisture content by 1 per cent. The variability of the parental rows, however, prevents an interpretation

of the mode of inheritance of moisture content in this experiment. The difficulty of determining the inheritance of moisture content, one of the qualities influencing hardiness, the results of which determination were shown in Table 19, tends to show that hardiness itself is not readily analyzed genetically.

TABLE 19.—*Frequency distribution of the moisture contents of plants of Kanred, Minhardi, and Kanred–Minhardi F_3 hybrid winter wheats at University Farm St. Paul, Minn., in June, 1924*

Moisture content classes	Number of rows		
	Kanred	Minhardi	F_3 hybrid lines
73.0–73.9 per cent			2
74.0–74.9 per cent		2	0
75.0–75.9 per cent		1	10
76.0–76.9 per cent	1	7	32
77.0–77.9 per cent	2	6	33
78.0–78.9 per cent	6	2	35
79.0–79.9 per cent	4	1	30
80.0–80.9 per cent	5		9
81.0–81.9 per cent	1		1
82.0–82.9 per cent			
83.0–83.9 per cent			
84.0–84.9 per cent			
85.0–85.9 per cent			
Total	19	19	152
Mean moisture content	79.12±0.18	76.91±0.21	77.87±0.08

DISCUSSION

The results here presented show many irregularities which, of course, make the drawing of accurate conclusions more difficult than with more uniform data. The studies, however, have brought out many points which must be taken into consideration in formulating the principles involved in hardiness in wheats. Using these studies, as well as the investigations of others, as a basis, the characteristics of hardiness and hardening in wheats and the factors influencing them may be considered.

CHARACTERISTICS OF HARDY VARIETIES

When growing actively in the fall or spring, hardy varieties of wheat as compared with nonhardy varieties are characterized by low moisture content and juice having a high percentage of total solids and a high freezing-point depression. The freezing-point depression and total solids in the juice are closely correlated, and both vary inversely with the moisture content of the tissue. Any variety of winter wheat in an unhardened condition will survive a temperature of −5° C. for 24 hours, but none will survive −10° C. for the same period. A hardening process is necessary, therefore, to permit wheat to survive the low winter temperatures which occur in the Northern States. Wheat plants without snow protection have survived temperatures of −25° C. or lower.

After hardening has occurred, the previously mentioned differences in moisture content and juice concentration between hardy and nonhardy varieties may not hold, or the relationship may be reversed. The hardy and nonhardy varieties then differ chiefly in imbibitional pressure of the cell colloids, as determined by the ability of wheat leaves to hold their moisture against the forces of

freezing and pressure. Hardy rye in an unhardened condition has a moisture content and juice concentration similar to those of a nonhardy wheat, but when hardened it has a greater imbibition pressure than the hardiest wheat.

At low temperatures, hardy varieties of rye and wheat have a lower rate of respiration than nonhardy wheats and thus retain their sugars longer or to a greater extent.

CHANGES OCCURRING DURING HARDENING

When growth is retarded by low temperature the sap concentration increases, due to the formation of sugars from starch and, after freezing, to the splitting of proteins into amino acids, as shown by Newton.[7] The sugars and some salts partly protect the protoplasmic colloids from being coagulated by freezing, according to Newton[7] (*35*, *36*) Maximow (*27*), Schaffnit (*48*), and Schander and Schaffnit (*50*). Freezing of the plants causes water to be withdrawn from the cells. Harvey (*19*) found that on thawing much of this water is evaporated, leaving the cell sap more concentrated than before freezing. Thus the moisture content of wheat leaves decreases during the autumn. With a decrease in moisture content and an increase in sugars and amino acids, the total solids increase and the freezing point of the juice is lowered. This lowering of the freezing point is of little consequence in itself, but the increase in solutes decreases the formation of ice and may protect the protoplasm from precipitation.

When the sap concentration increases, the resistance to freezing of the plant tissue increases at a still greater rate, as noted by Chandler (*9*). Apparently the increased juice concentration results in an increased imbibition pressure of the cell colloids, which decreases the withdrawal of water from the cells by freezing or pressure. Probably the increased imbibition pressure is the result of greater dispersion of the protoplasmic proteins. According to Petit (*41*), imbibed or capillary water permits more undercooling before ice is formed.

Hardy varieties having a greater initial juice concentration possess a slightly greater resistance to freezing injury, probably because less water is withdrawn from the cell in freezing. At low temperatures, hardy varieties form more sugar and increase the sap concentration more than nonhardy varieties. More of the plant tissues of hardy varieties also remain active after a freeze, even with low moisture content. The greater juice concentration in the living cells of the hardy plants causes a greater imbibition of cell juice by protoplasmic colloids. This, together with the greater protection against coagulation of the protoplasm by freezing, due to sugars, accounts for the cold resistance of certain varieties of wheat and rye. The more hardy the variety the more readily is it able to increase the imbibition pressure of the tissues.

The temperatures or combination of temperatures best suited for hardening in wheat are not known, and their determination should furnish a fertile subject for investigation. Results reported here show that gradual reductions in temperature increase hardiness and that exposure to 0° C. is more effective than exposure to temperatures

[7] NEWTON, R. Op. cit.

of 5° C. or −5° C. Probably alternations of freezing and thawing are most effective.

The time required for maximum hardening also is not known. Rein (*42*) reported that maximum hardening occurred in some plants in 6 to 8 days. Winkler (*57*) reported that trees can be hardened by a 5-day exposure to low temperature even in the summer.

When the temperature rises above freezing, water can enter the plants and also the cells if the protoplasm has not been killed. This increase in moisture decreases the juice concentration. At a sufficiently high temperature the sugars will revert to starch and the amino acids will form proteins. The imbibition pressure will then decrease, and the tissues will be less hardy. Rather marked differences have been observed between successive collections made three or four days apart. A decided difference in rye was observed between the collections of November 17 and 18, 1925, and a still more abrupt difference was observed between the Kanred wheat collected in the morning and that collected in the afternoon of November 5. However, the later collections of both rye and wheat were in the laboratory three or four hours while the plant parts were being separated, which might account for some of the changes in these instances.

A freeze during the night, followed by thawing, will increase the quantity of juice that can be expressed from the leaves. After the evaporation of the melted ice crystals, less juice can be expressed. If a freeze is not too severe, the plant hardens upon thawing, but after a hard freeze the protoplasm is coagulated and dehydrated to such an extent that it can not take up water and the plant is killed instead of hardened.

With different freezing temperatures affecting hardy and nonhardy varieties somewhat differently, and with various degrees of recovery or response to temperatures above freezing, the irregularity in the winterkilling of wheats is not surprising. Adjoining rows of wheat show rather wide differences in the imbibition pressure of the tissues. Doubtless there are nearly as great differences between plants within a row. If so, this further explains the irregularities in killing which usually occur and which can not be explained by differences in protection of the plants.

THE PRACTICAL MEASUREMENT OF HARDINESS

The results of these studies indicate that no laboratory method yet devised, except perhaps the controlled freezing, is any more accurate for determining hardiness than is careful field study. The moisture content of unwilted wheat leaves probably is only one of the factors concerned in hardiness and is itself subject to considerable variation. When wilted tissue is present, moisture content is not a dependable character, as tender varieties are wilted more than hardy ones. Under ideal conditions, however, the moisture content is a valuable index of hardiness in wheat leaves.

The imbibition pressure in wheat leaves may show a striking relationship to hardiness during late autumn, but even then it is highly variable from day to day. When the tissue remains frozen during the winter, or when the plants freeze while in a growing condition, the imbibition pressure, as measured by the quantity of expressed juice, is of little value in determining hardiness. This test requires

considerable labor and equipment at all times; therefore, in view of its variability, it can not be expected to serve as a practical measure of hardiness in wheat except under ideal conditions such as are obtained in certain seasons.

Total solids, freezing-point depression, and bound water in the juice are related to hardiness only under certain conditions and are of little use for practical measurement of hardiness except early in the autumn, when the total solids and freezing-point depression are inversely proportional to hardiness. The bound-water content of the juice from macerated tissue should measure the imbibition of the protoplasmic colloids, but its determination is more tedious than that of ordinary freezing and pressing.

Freezing under controlled temperatures offers the greatest promise in measuring the hardiness of wheat plants by laboratory methods, even though the cold resistance of the plants does vary considerably. A rough measure of hardiness can be obtained without any previous hardening, but for more accurate measurements the plants must be subjected to successively lower temperatures. Refrigeration equipment, although expensive, probably would be the most economical means of accurately measuring cold resistance, if an exact technic in hardening were developed. The next most feasible method is by growing the varieties in replicated rows at selected field stations. The plants can be protected somewhat by sowing the seed between rows of grain stubble where conditions are rather severe, or protection can be decreased by thin sowing.

SUMMARY

The studies here presented are concerned only with low temperatures and not with killing due to winter drought, soil blowing, heaving, or smothering by ice.

Hardy wheats are characterized by a low moisture content of the tissues, a high percentage of total solids in the juice, a high freezing-point depression or osmotic concentration of the juice when the plants are actively growing, a high percentage of bound water in the juice, a low rate of respiration at low temperatures, and frequently by a long period of vegetative growth.

The most important character influencing hardiness is the ability to build up a high imbibition pressure of the cell colloids during hardening.

Swedish (Minnesota No. 2) rye, which is more hardy than any wheat, has a high moisture content, a low percentage of total solids, and a low freezing-point depression like nonhardy wheats, but it has a lower rate of respiration at low temperatures and a greater imbibition pressure than the hardiest wheats.

During the hardening of wheats there is a decrease in moisture content and an increase in total solids in the sap, freezing-point depression of the sap, and imbibition pressure of the cell colloids, as measured by the ability of the tissues to hold sap against the forces of freezing and pressure.

In the tissues of a given variety, the quantity of juice expressed is positively correlated with the moisture content. The percentage of total solids and the freezing-point depression of the juice are negatively correlated with moisture content and the quantity of

juice expressed from the tissues. The quantity of juice retained after pressing is positively correlated with moisture content in unfrozen tissues, but not significantly so in frozen tissues.

The moisture content, sap concentration, imbibition pressure, and hardiness of wheat and rye plants fluctuate widely during the fall and winter, and differences between varieties do not exist at all times.

The freezing of plants at controlled temperatures, followed by a determination of the extent of killing, appears to be the most feasible laboratory method for measuring hardiness.

A sudden exposure of wheat and rye plants from the greenhouse to a temperature of −10° C. for 24 hours will kill the hardiest varieties. Any variety of winter wheat will withstand a temperature of −5° C. for the same period. Hardening is necessary to protect wheat against the much lower temperatures than −10° C. which occur in northern wheat-growing areas. The hardier the variety the more it can harden.

The crown is the most hardy portion of the wheat plant above the soil surface. Young leaves are more hardy than old leaves, and the bases of leaves are more hardy than the tips.

The genetic factors involved in the inheritance of hardiness have not been determined, but most F_3 strains are intermediate between the parents in hardiness. Accurate measures of hardiness in the field are difficult because of the variations both in the plants and in weather conditions.

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